LOGINID:ssspta1617mxb

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NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Apr 08	"Ask CAS" for self-help around the clock
NEWS 3	Jun 03	New e-mail delivery for search results now available
NEWS 4	Aug 08	PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 5	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 6	Aug 26	Sequence searching in REGISTRY enhanced
NEWS 7	Sep 03	JAPIO has been reloaded and enhanced
NEWS 8	Sep 16	Experimental properties added to the REGISTRY file
NEWS 9	Sep 16	CA Section Thesaurus available in CAPLUS and CA
NEWS 10	Oct 01	CASREACT Enriched with Reactions from 1907 to 1985
NEWS 11	Oct 24	BEILSTEIN adds new search fields
NEWS 12	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 13	Nov 18	DKILIT has been renamed APOLLIT
NEWS 14	Nov 25	More calculated properties added to REGISTRY
NEWS 15	Dec 04	CSA files on STN
NEWS 16	Dec 17	PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS 17	Dec 17	TOXCENTER enhanced with additional content
NEWS 18	Dec 17	Adis Clinical Trials Insight now available on STN
NEWS 19	Jan 29	Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC
NEWS 20	Feb 13	CANCERLIT is no longer being updated
NEWS 21	Feb 24	METADEX enhancements
NEWS 22	Feb 24	PCTGEN now available on STN
NEWS 23	Feb 24	TEMA now available on STN
NEWS 24	Feb 26	NTIS now allows simultaneous left and right truncation
NEWS 25	Feb 26	PCTFULL now contains images
NEWS 26	Mar 04	SDI PACKAGE for monthly delivery of multifile SDI results
NEWS 27	Mar 20	EVENTLINE will be removed from STN
NEWS 28	Mar 24	PATDPAFULL now available on STN
NEWS 29	Mar 24	Additional information for trade-named substances without structures available in REGISTRY
NEWS 30	Apr 11	Display formats in DGENE enhanced
NEWS 31	Apr 14	MEDLINE Reload
NEWS 32	Apr 17	Polymer searching in REGISTRY enhanced
NEWS 33	Apr 21	Indexing from 1947 to 1956 being added to records in CA/CAPLUS
NEWS 34	Apr 21	New current-awareness alert (SDI) frequency in WPIDS/WPINDEX/WPIX
NEWS 35	Apr 28	RDISCLOSURE now available on STN
NEWS 36	May 05	Pharmacokinetic information and systematic chemical names added to PHAR
NEWS 37	May 15	MEDLINE file segment of TOXCENTER reloaded
NEWS 38	May 15	Supporter information for ENCOMPPAT and ENCOMPLIT updated
NEWS 39	May 16	CHEMREACT will be removed from STN

NEWS EXPRESS	April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information

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  NEWS LOGIN     Welcome Banner and News Items
  NEWS PHONE     Direct Dial and Telecommunication Network Access to STN
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FILE 'HOME' ENTERED AT 17:52:21 ON 16 MAY 2003

=> fil caplus medline uspatfull
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                          ENTRY     SESSION
FULL ESTIMATED COST                                        0.21        0.21

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FILE 'MEDLINE' ENTERED AT 17:52:49 ON 16 MAY 2003

FILE 'USPATFULL' ENTERED AT 17:52:49 ON 16 MAY 2003
CA INDEXING COPYRIGHT (C) 2003 AMERICAN CHEMICAL SOCIETY (ACS)

=> s azathioprine or mercaptopurine or thioguanine
L1         33004 AZATHIOPRINE OR MERCAPTOPURINE OR THIOGUANINE

=> s aphthous or GVHD or pemphigus vulgaris or pemphigoid or aphthae
L2         18176 APHTHOUS OR GVHD OR PEMPHIGUS VULGARIS OR PEMPHIGOID OR APHTHAE

=> s l1 and l2
L3           992 L1 AND L2

=> s l3 and py<1999
L4           296 L3 AND PY<1999

=>
=> s azathioprine
L5         17693 AZATHIOPRINE

=> s l4 and l5
L6           272 L4 AND L5

=> s topical or lollipop or pellet or gel or ointment or cream or lozenge
L7       1294788 TOPICAL OR LOLLIPOP OR PELLET OR GEL OR OINTMENT OR CREAM OR
                 LOZENGE

=> s l6 and l7
L8            87 L6 AND L7

=> s l8 and py<1998
L9            68 L8 AND PY<1998
```

=> d l9 1-68 ab bib kwic

L9 ANSWER 1 OF 68 CAPLUS COPYRIGHT 2003 ACS
AB UVB irradn. of bone marrow or pancreatic islets has been shown to prevent **GVHD** and to induce transplant tolerance in exptl. animal models. To clarify the underlying mechanism(s) responsible for these UVB effects we have examd. in vitro cell function following UVB irradn. using LDA, FACS anal., and DNA **gel** electrophoresis to assess the role of UVB-induced anergy and/or cell death. To extend our studies to the clin. setting and to promote chimerism and tolerance to organ allografts, we have further studied the effects of UVB irradn. combined with the commonly used immunosuppressive agents (cyclosporine, **azathioprine**, and methylprednisolone) on human T-cells in proliferative in vitro assays. When cytotoxic and proliferative responses to allogeneic cells or to PHA stimulation were evaluated in LDA, the use of increasing doses of UVB irradn. resulted in a dose-dependent decrease in proliferative and cytotoxic responses of T-cells as seen by decreases in precursor frequencies. The results of proliferative T-cell assays suggest an additive immunosuppressive effect of various immunosuppressive drugs when combined with UVB irradn. **Gel** electrophoresis of DNA derived from resting and activated, UVB-irradiated PBLs showed apoptosis at all UVB doses used. FACS anal. of UVB-treated CD2+ cells resulted in a UVB dose-related decrease in cell nos. that correlated with viability studies. It appears that UVB irradn. of both activated and resting PBLs induces programmed cell death but not anergy of T-cells.
AN 1996:176906 CAPLUS
DN 124:254647
TI UVB irradiation of human-derived peripheral blood lymphocytes induces apoptosis but not T-cell anergy: additive effects with various immunosuppressive agents
AU Yaron, Iris; Yaron, Renat; Oluwole, Soji F.; Hardy, Mark A.
CS Dep. Surgery, Columbia Univ. Coll. Physicians Surgeons, New York, NY, 10032, USA
SO Cellular Immunology (**1996**), 168(2), 258-66
CODEN: CLIMB8; ISSN: 0008-8749
PB Academic
DT Journal
LA English
SO Cellular Immunology (**1996**), 168(2), 258-66
CODEN: CLIMB8; ISSN: 0008-8749
AB UVB irradn. of bone marrow or pancreatic islets has been shown to prevent **GVHD** and to induce transplant tolerance in exptl. animal models. To clarify the underlying mechanism(s) responsible for these UVB effects we have examd. in vitro cell function following UVB irradn. using LDA, FACS anal., and DNA **gel** electrophoresis to assess the role of UVB-induced anergy and/or cell death. To extend our studies to the clin. setting and to promote chimerism and tolerance to organ allografts, we have further studied the effects of UVB irradn. combined with the commonly used immunosuppressive agents (cyclosporine, **azathioprine**, and methylprednisolone) on human T-cells in proliferative in vitro assays. When cytotoxic and proliferative responses to allogeneic cells or to PHA stimulation were evaluated in LDA, the use of increasing doses of UVB irradn. resulted in a dose-dependent decrease in proliferative and cytotoxic responses of T-cells as seen by decreases in precursor frequencies. The results of proliferative T-cell assays suggest an additive immunosuppressive effect of various immunosuppressive drugs when

combined with UVB irradn. **Gel** electrophoresis of DNA derived from resting and activated, UVB-irradiated PBLs showed apoptosis at all UVB doses used. FACS anal. of UVB-treated CD2+ cells resulted in a UVB dose-related decrease in cell nos. that correlated with viability studies.

It appears that UVB irradn. of both activated and resting PBLs induces programmed cell death but not anergy of T-cells.

IT 83-43-2, Methylprednisolone 446-86-6, **Azathioprine** 59865-13-3, Cyclosporine

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(UVB irradn. of human-derived peripheral blood lymphocytes induces apoptosis but not T-cell anergy: additive effects with various immunosuppressants)

L9 ANSWER 2 OF 68 MEDLINE

AB UVB irradiation of bone marrow or pancreatic islets has been shown to prevent **GVHD** and to induce transplant tolerance in experimental animal models. To clarify the underlying mechanism(s) responsible for these UVB effects we have examined in vitro cell function following UVB irradiation using LDA, FACS analysis, and DNA **gel** electrophoresis to assess the role of UVB-induced anergy and/or cell death. To extend our studies to the clinical setting and to promote chimerism and tolerance to organ allografts, we have further studied the effects of UVB irradiation combined with the commonly used immunosuppressive agents (cyclosporine, **azathioprine**, and methylprednisolone) on human T cells in proliferative in vitro assays. When cytotoxic and proliferative responses to allogeneic cells or to PHA stimulation were evaluated in LDA, the use of increasing doses of UVB irradiation resulted in a dose-dependent decrease in proliferative and cytotoxic responses of T-cells as seen by decreases in precursor frequencies. The results of proliferative T-cell assays suggest an additive immunosuppressive effect of various immunosuppressive drugs when combined with UVB irradiation. **Gel** electrophoresis of DNA derived from resting and activated, UVB-irradiated PBLs showed apoptosis at all UVB doses used. FACS analysis of UVB-treated CD2+ cells resulted in a UVB dose-related decrease in cell numbers that correlated with viability studies. It appears that UVB irradiation of both activated and resting PBLs induces programmed cell death but not anergy of T-cells.

AN 96228283 MEDLINE

DN 96228283 PubMed ID: 8640873

TI UVB irradiation of human-derived peripheral blood lymphocytes induces apoptosis but not T-cell anergy: additive effects with various immunosuppressive agents.

AU Yaron I; Yaron R; Oluwole S F; Hardy M A

CS Department of Surgery, Columbia University College of Physicians and Surgeons, New York 10032, USA.

NC CA52678 (NCI)
HL14799 (NHLBI)

SO CELLULAR IMMUNOLOGY, **(1996 Mar 15)** 168 (2) 258-66.
Journal code: 1246405. ISSN: 0008-8749.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199607

ED Entered STN: 19960726

Last Updated on STN: 19960726
Entered Medline: 19960717
SO CELLULAR IMMUNOLOGY, **(1996 Mar 15)** 168 (2) 258-66.
Journal code: 1246405. ISSN: 0008-8749.
AB UVB irradiation of bone marrow or pancreatic islets has been shown to prevent **GVHD** and to induce transplant tolerance in experimental animal models. To clarify the underlying mechanism(s) responsible for these UVB effects we have examined in vitro cell function following UVB irradiation using LDA, FACS analysis, and DNA **gel** electrophoresis to assess the role of UVB-induced anergy and/or cell death. To extend our studies to the clinical setting and. . . to organ allografts, we have further studied the effects of UVB irradiation combined with the commonly used immunosuppressive agents (cyclosporine, **azathioprine**, and methylprednisolone) on human T cells in proliferative in vitro assays. When cytotoxic and proliferative responses to allogeneic cells or. . . The results of proliferative T-cell assays suggest an additive immunosuppressive effect of various immunosuppressive drugs when combined with UVB irradiation. **Gel** electrophoresis of DNA derived from resting and activated, UVB-irradiated PBLs showed apoptosis at all UVB doses used. FACS analysis of. . .
CT . . . Check Tags: Comparative Study; Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Apoptosis: DE, drug effects
*Apoptosis: RE, radiation effects
***Azathioprine: PD, pharmacology**
Clonal Anergy: DE, drug effects
Clonal Anergy: RE, radiation effects
*Cyclosporine: PD, pharmacology
Cytotoxicity, Immunologic: DE, drug effects
. . .
RN **446-86-6 (Azathioprine)**; 59865-13-3 (Cyclosporine); 83-43-2 (Methylprednisolone)

L9 ANSWER 3 OF 68 MEDLINE
AB OBJECTIVE: To present the first case of bullous **pemphigoid** in an Australian Aborigine. CLINICAL FEATURES: A 47 year old female aborigine presented with a three week history of a generalised skin eruption consistent with bullous **pemphigoid**. Immunohistological examination confirmed the diagnosis. Therapy required high dose oral steroids, **azathioprine** and erythromycin as well as **topical** agents. Treatment was complicated by isolation and poor compliance but was ultimately successful in inducing and retaining remission. CONCLUSION: This is the first description of bullous **pemphigoid** in an Australian Aborigine. We recommend early biopsy to confirm diagnosis and plan therapy, and careful attention to patient education to encourage compliance.
AN 94145470 MEDLINE
DN 94145470 PubMed ID: 8311825
TI Bullous **pemphigoid** in an Aborigine.
AU Lo S; Mollison L C; Kumar A
CS Alice Springs Hospital, Australia.
SO AUSTRALASIAN JOURNAL OF DERMATOLOGY, **(1993)** 34 (2) 41-4.
Journal code: 0135232. ISSN: 0004-8380.
CY Australia
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199403

ED Entered STN: 19940330
Last Updated on STN: 19940330
Entered Medline: 19940315
TI Bullous **pemphigoid** in an Aborigine.
SO AUSTRALASIAN JOURNAL OF DERMATOLOGY, **(1993)** 34 (2) 41-4.
Journal code: 0135232. ISSN: 0004-8380.
AB OBJECTIVE: To present the first case of bullous **pemphigoid** in an Australian Aborigine. CLINICAL FEATURES: A 47 year old female aborigine presented with a three week history of a generalised skin eruption consistent with bullous **pemphigoid**. Immunohistological examination confirmed the diagnosis. Therapy required high dose oral steroids, **azathioprine** and erythromycin as well as **topical** agents. Treatment was complicated by isolation and poor compliance but was ultimately successful in inducing and retaining remission. CONCLUSION: This is the first description of bullous **pemphigoid** in an Australian Aborigine. We recommend early biopsy to confirm diagnosis and plan therapy, and careful attention to patient education. . .
CT Check Tags: Case Report; Female; Human
*Aborigines
Australia: EP, epidemiology
Middle Age
***Pemphigoid, Bullous**
Pemphigoid, Bullous: EH, ethnology
Pemphigoid, Bullous: PA, pathology

L9 ANSWER 4 OF 68 MEDLINE
AB The initial oral findings and treatment in 50 cases of mucous membrane **pemphigoid** are presented. Histologic and immunologic studies were undertaken in each case to confirm the clinical diagnosis. The treatments prescribed are summarized and illustrate that **topical** steroids are effective, but in some cases systemic steroid therapy with or without other immunologically active drugs is required. A significant number of patients had extraoral manifestations of the disorder.
AN 92375485 MEDLINE
DN 92375485 PubMed ID: 1508509
TI Mucous membrane **pemphigoid**. Treatment experience at two institutions.
AU Lamey P J; Rees T D; Binnie W H; Rankin K V
CS Department of Oral Medicine, Glasgow Dental Hospital and School, Scotland.
SO ORAL SURGERY, ORAL MEDICINE, AND ORAL PATHOLOGY, **(1992 Jul)** 74 (1) 50-3.
Journal code: 0376406. ISSN: 0030-4220.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Dental Journals; Priority Journals
EM 199209
ED Entered STN: 19921009
Last Updated on STN: 19921009
Entered Medline: 19920918
TI Mucous membrane **pemphigoid**. Treatment experience at two institutions.
SO ORAL SURGERY, ORAL MEDICINE, AND ORAL PATHOLOGY, **(1992 Jul)** 74 (1) 50-3.
Journal code: 0376406. ISSN: 0030-4220.
AB The initial oral findings and treatment in 50 cases of mucous membrane **pemphigoid** are presented. Histologic and immunologic studies were

undertaken in each case to confirm the clinical diagnosis. The treatments
prescribed are summarized and illustrate that **topical** steroids
are effective, but in some cases systemic steroid therapy with or without
other immunologically active drugs is required. A. . .

CT Check Tags: Female; Human; Male
Adolescent
Adult
Aged
Azathioprine: TU, therapeutic use
Cyclophosphamide: TU, therapeutic use
Dapsone: TU, therapeutic use
Gingival Diseases: DT, drug therapy
Glucocorticoids, Topical: TU, therapeutic use
Middle Age
Mouth Diseases: DT, drug therapy
Mouth Mucosa
***Pemphigoid, Benign Mucous Membrane: DT, drug therapy**
Prednisolone: TU, therapeutic use

RN **446-86-6 (Azathioprine)**; 50-18-0 (Cyclophosphamide); 50-24-8 (Prednisolone); 80-08-0 (Dapsone)

CN 0 (Glucocorticoids, **Topical**)

L9 ANSWER 5 OF 68 MEDLINE

AB Cicatricial **pemphigoid** is a subepidermal blistering disease that involves the mucous membranes and the skin. The oral cavity and the eye are most frequently involved. The clinical course is of long duration, and often there is significant scarring that can have devastating sequelae. The majority of the patients are elderly. The disease is characterized by the in vivo deposition of an anti-basement membrane zone antibody. The anti-basement membrane zone antibody cannot be detected in the circulation by routine laboratory techniques. The pathogenesis is poorly understood, and the cause is not known. Cicatricial **pemphigoid** may remain localized to the oral cavity or the eye or the skin (Brunsting-Perry variety), or it may be generalized. It rarely occurs in children, and it may be drug induced. Efforts must be made to differentiate cicatricial **pemphigoid** from bullous **pemphigoid**, epidermolysis bullosa acquisita, linear IgA bullous disease, and other vesiculobullous disease. Early recognition and treatment can improve the prognosis and avoid surgical intervention. **Topical** therapy is beneficial and expedites healing. Intralesional corticosteroids are effective and can help reduce the dose of systemic steroids. Most patients require systemic corticosteroid therapy. Dapsone is also useful in treating cicatricial **pemphigoid**, especially in patients in whom systemic steroids are ineffective or in whom they have to be discontinued because of side effects. Immunosuppressive agents (**azathioprine** or cyclosphosphamide) are indicated in patients with progressive disease. Occasionally both drugs may be needed.

AN 91332268 MEDLINE

DN 91332268 PubMed ID: 1869688

TI Cicatricial **pemphigoid**.

CM Comment in: J Am Acad Dermatol. 1993 Jan;28(1):134-5

AU Ahmed A R; Kurgis B S; Rogers R S 3rd

CS Center for Blood Research, Boston, MA 02115.

NC 1R01 EY08379-01 (NEI)

SO JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY, **(1991 Jun)** 24 (6 Pt 1) 987-1001. Ref: 96
Journal code: 7907132. ISSN: 0190-9622.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199109
ED Entered STN: 19911006
Last Updated on STN: 19911006
Entered Medline: 19910919
TI Cicatricial **pemphigoid**.
SO JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY, **(1991 Jun)** 24 (6 Pt 1) 987-1001. Ref: 96
Journal code: 7907132. ISSN: 0190-9622.
AB Cicatricial **pemphigoid** is a subepidermal blistering disease that involves the mucous membranes and the skin. The oral cavity and the eye are. . . detected in the circulation by routine laboratory techniques. The pathogenesis is poorly understood, and the cause is not known. Cicatricial **pemphigoid** may remain localized to the oral cavity or the eye or the skin (Brunsting-Perry variety), or it may be generalized. It rarely occurs in children, and it may be drug induced. Efforts must be made to differentiate cicatricial **pemphigoid** from bullous **pemphigoid**, epidermolysis bullosa acquisita, linear IgA bullous disease, and other vesiculobullous disease. Early recognition and treatment can improve the prognosis and avoid surgical intervention. **Topical** therapy is beneficial and expedites healing. Intralesional corticosteroids are effective and can help reduce the dose of systemic steroids. Most patients require systemic corticosteroid therapy. Dapsone is also useful in treating cicatricial **pemphigoid**, especially in patients in whom systemic steroids are ineffective or in whom they have to be discontinued because of side effects. Immunosuppressive agents (**azathioprine** or cyclosphosphamide) are indicated in patients with progressive disease. Occasionally both drugs may be needed.
CT Check Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
***Pemphigoid, Benign Mucous Membrane**
Pemphigoid, Benign Mucous Membrane: DT, drug therapy
Pemphigoid, Benign Mucous Membrane: PA, pathology

L9 ANSWER 6 OF 68 MEDLINE
AB Bullous **pemphigoid** (BP) and benign mucous membrane **pemphigoid** (BMMP) are autoimmune diseases characterised by subepithelial bulla formation and showing substantial overlap in clinical signs and symptoms. BP principally involves skin and BMMP the oral mucosa and eyes. The gingiva are affected in 90% of cases of BMMP and buccal mucosa and palate in up to 30%. Lesions may heal with scarring. Extension into the pharynx and esophagus causes sore throat and dysphagia. Severe ocular involvement may cause blindness. Bulla formation is attributed to complement activation, following IgG binding to the basement membrane zone, with subsequent polymorphonuclear leukocyte accumulation. The target antigen in BP is a 180-230 kD protein associated with the basilar membrane of basal keratinocytes. The gene encoding the BP antigen has been partially cloned. It is likely that the same antigen is involved in BMMP, but the mechanism of scarring is not understood. Treatment of BP

and BMMP includes systemic steroid and **azathioprine** therapy and **topical** steroids.

AN 90188943 MEDLINE
DN 90188943 PubMed ID: 2179535
TI Vesiculo-bullous mucocutaneous disease: benign mucous membrane and bullous **pemphigoid**.
AU Williams D M
CS Department of Oral Pathology, London Hospital Medical College, England.
SO JOURNAL OF ORAL PATHOLOGY AND MEDICINE, **(1990 Jan)** 19 (1) 16-23.
Ref: 94
Journal code: 8911934. ISSN: 0904-2512.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Dental Journals; Priority Journals
EM 199004
ED Entered STN: 19900601
Last Updated on STN: 19900601
Entered Medline: 19900419
TI Vesiculo-bullous mucocutaneous disease: benign mucous membrane and bullous **pemphigoid**.
SO JOURNAL OF ORAL PATHOLOGY AND MEDICINE, **(1990 Jan)** 19 (1) 16-23.
Ref: 94
Journal code: 8911934. ISSN: 0904-2512.
AB Bullous **pemphigoid** (BP) and benign mucous membrane **pemphigoid** (BMMP) are autoimmune diseases characterised by subepithelial bulla formation and showing substantial overlap in clinical signs and symptoms. BP principally. . . involved in BMMP, but the mechanism of scarring is not understood. Treatment of BP and BMMP includes systemic steroid and **azathioprine** therapy and **topical** steroids.
CT . . .
Diseases: PA, pathology
Basement Membrane: PA, pathology
Corneal Ulcer: PA, pathology
Diagnosis, Differential
*Gingivitis: PA, pathology
Mouth Mucosa: PA, pathology
***Pemphigoid, Benign Mucous Membrane**
Pemphigoid, Benign Mucous Membrane: PA, pathology
***Pemphigoid, Bullous**
Pemphigoid, Bullous: PA, pathology
*Skin Diseases, Vesiculobullous
Skin Diseases, Vesiculobullous: PA, pathology

L9 ANSWER 7 OF 68 MEDLINE
AB A 32-year-old woman with a 3-month history of severe major **aphthous** stomatitis covering the anterior dorsal third of the tongue was treated successfully with **topical** dexamethasone mouthrinse and oral **azathioprine** tablets. The lesion was resolved within 90 days without side effects.
AN 90114927 MEDLINE
DN 90114927 PubMed ID: 2296449
TI Combination immunosuppressant and **topical** steroid therapy for treatment of recurrent major **aphthae**. A case report.
AU Brown R S; Bottomley W K

CS Department of Oral Diagnostic Sciences, University of Texas Health Science
Center, Houston.
SO ORAL SURGERY, ORAL MEDICINE, AND ORAL PATHOLOGY, **(1990 Jan)** 69 (1) 42-4.
Journal code: 0376406. ISSN: 0030-4220.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Dental Journals; Priority Journals
EM 199002
ED Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19900222
TI Combination immunosuppressant and **topical** steroid therapy for treatment of recurrent major **aphthae**. A case report.
SO ORAL SURGERY, ORAL MEDICINE, AND ORAL PATHOLOGY, **(1990 Jan)** 69 (1) 42-4.
Journal code: 0376406. ISSN: 0030-4220.
AB A 32-year-old woman with a 3-month history of severe major **aphthous** stomatitis covering the anterior dorsal third of the tongue was treated successfully with **topical** dexamethasone mouthrinse and oral **azathioprine** tablets. The lesion was resolved within 90 days without side effects.
CT Check Tags: Case Report; Female; Human
Adult
*Autoimmune Diseases: DT, drug therapy
***Azathioprine: TU, therapeutic use**
*Dexamethasone: TU, therapeutic use
Ibuprofen: TU, therapeutic use
***Stomatitis, Aphthous: DT, drug therapy**
*Tongue Diseases: DT, drug therapy
RN 15687-27-1 (Ibuprofen); **446-86-6 (Azathioprine)**; 50-02-2 (Dexamethasone)

L9 ANSWER 8 OF 68 MEDLINE
AB Epidermolysis bullosa acquisita is a chronic, severe, subepidermal, blistering disease of the skin, characterized by marked resistance to **topical** and systemic therapy. This report concerns a well-documented case of a woman who had had epidermolysis bullosa acquisita for 6 years and had remained hospitalized continuously for 7 months in 1987. Her case ultimately was controlled with cyclosporine after the failure of a variety of therapeutic modalities in the hospital, including prednisone, methotrexate, **azathioprine**, phenytoin, vitamin E, gold sodium thiomalate (Myochrysine), isotretinoin, and plasmapheresis. In contrast to patients with pemphigus and **pemphigoid** treated with cyclosporine, our patient's autoantibodies did not disappear on therapy. Although its mechanism of action in epidermolysis bullosa acquisita is unknown, we propose that cyclosporine may be a helpful drug for patients whose disease is refractory to more traditional forms of therapy.
AN 89054517 MEDLINE
DN 89054517 PubMed ID: 3057000
TI Clearing of epidermolysis bullosa acquisita with cyclosporine.
CM Comment in: J Am Acad Dermatol. 1990 Mar;22(3):535-6
Comment in: J Am Acad Dermatol. 1991 Jun;24(6 Pt 1):1034-5
AU Crow L L; Finkle J P; Gammon W R; Woodley D T
CS Department of Dermatology, University of North Carolina School of Medicine, Chapel Hill 27514.
NC AM33625 (NIADDK)

AR30475 (NIAMS)
SO JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY, **(1988 Nov)** 19 (5 Pt 2) 937-42.
Journal code: 7907132. ISSN: 0190-9622.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198901
ED Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19890103
SO JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY, **(1988 Nov)** 19 (5 Pt 2) 937-42.
Journal code: 7907132. ISSN: 0190-9622.
AB Epidermolysis bullosa acquisita is a chronic, severe, subepidermal, blistering disease of the skin, characterized by marked resistance to **topical** and systemic therapy. This report concerns a well-documented case of a woman who had had epidermolysis bullosa acquisita for 6. . . ultimately was controlled with cyclosporine after the failure of a variety of therapeutic modalities in the hospital, including prednisone, methotrexate, **azathioprine**, phenytoin, vitamin E, gold sodium thiomalate (Myochrysine), isotretinoin, and plasmapheresis. In contrast to patients with pemphigus and **pemphigoid** treated with cyclosporine, our patient's autoantibodies did not disappear on therapy. Although its mechanism of action in epidermolysis bullosa acquisita. . .

L9 ANSWER 9 OF 68 MEDLINE
AB **Pemphigus vulgaris**, whether of the vulgaris or foliaceus variety, and bullous **pemphigoid** (BP) are two groups of auto-immune bullous diseases which in most cases can easily be differentiated on the basis of clinical, histological and, mainly, immunopathological data. Like cicatricial **pemphigoid**, BP may be accompanied with circulating pemphigus-like antibodies (PLA) which are not detected in vivo by direct immunofluorescence (IF). However, a true pemphigus-BP association, as reported first by Chorzelski et al., is exceptional. Two cases of BP immunolabelled with pemphigus-like antibodies at direct IF are reported, raising a discussion on this particular association. The first case concerns a 62-year old man presenting with extensive psoriasis treated with salicylated vaseline and **topical** corticosteroids. The patients was admitted for a disseminated, symmetrical and pruriginous bullous eruption made up of tense bullae on healthy and psoriatic skin or on an urticarial background, without Nikolsky's sign. Pathological examination of a recent bulla showed subepidermal detachment without acantholysis. Direct cutaneous IF revealed linear labelling of the basement membrane zone with IgG, C3 and C1q, and labelling of the inter-cellular substance of the epidermis with IgG. Indirect IF on O+ human skin demonstrated antibodies of the **pemphigoid** type (1/128) and of the pemphigus type (1/64). Standard laboratory examinations only showed moderate blood eosinophilia (950/mm3) and a rise in total IgE. Under systemic corticosteroid therapy (prednisone 1 mg/kg/day) and **azathioprine** (2 mg/kg/day) the bullae rapidly disappeared. (ABSTRACT TRUNCATED AT 250 WORDS)
AN 87098435 MEDLINE
DN 87098435 PubMed ID: 3541760
TI [Bullous **pemphigoid** with pemphigus type antibodies in vivo. 2 cases].

Pemphigoide bulleuse avec anticorps de type pemphigus in vivo. Deux observations.
AU Bernard P; Catanzano G; Vignaud St Florent J D; Fayol J; Bonnetblanc J M
SO ANNALES DE DERMATOLOGIE ET DE VENEREOLOGIE, **(1986)** 113 (8) 671-6.
Journal code: 7702013. ISSN: 0151-9638.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
LA French
FS Priority Journals
EM 198702
ED Entered STN: 19900302
Last Updated on STN: 19900302
Entered Medline: 19870212
TI [Bullous **pemphigoid** with pemphigus type antibodies in vivo. 2 cases].
Pemphigoide bulleuse avec anticorps de type pemphigus in vivo. Deux observations.
SO ANNALES DE DERMATOLOGIE ET DE VENEREOLOGIE, **(1986)** 113 (8) 671-6.
Journal code: 7702013. ISSN: 0151-9638.
AB **Pemphigus vulgaris**, whether of the vulgaris or foliaceus variety, and bullous **pemphigoid** (BP) are two groups of auto-immune bullous diseases which in most cases can easily be differentiated on the basis of clinical, histological and, mainly, immunopathological data. Like cicatricial **pemphigoid**, BP may be accompanied with circulating pemphigus-like antibodies (PLA) which are not detected in vivo by direct immunofluorescence (IF). However,. . . this particular association. The first case concerns a 62-year old man presenting with extensive psoriasis treated with salicylated vaseline and **topical** corticosteroids. The patients was admitted for a disseminated, symmetrical and pruriginous bullous eruption made up of tense bullae on healthy. . . labelling of the inter-cellular substance of the epidermis with IgG. Indirect IF on O+ human skin demonstrated antibodies of the **pemphigoid** type (1/128) and of the pemphigus type (1/64). Standard laboratory examinations only showed moderate blood eosinophilia (950/mm3) and a rise in total IgE. Under systemic corticosteroid therapy (prednisone 1 mg/kg/day) and **azathioprine** (2 mg/kg/day) the bullae rapidly disappeared.(ABSTRACT TRUNCATED AT 250 WORDS)
CT . . . Case Report; Female; Human; Male
Aged
Aged, 80 and over
*Antibodies: AN, analysis
English Abstract
Fluorescent Antibody Technique
Middle Age
***Pemphigoid, Bullous: IM, immunology**
Pemphigoid, Bullous: PA, pathology
*Pemphigus: IM, immunology
Pemphigus: PA, pathology
Psoriasis: PA, pathology
*Skin Diseases, Vesiculobullous: IM, immunology

L9 ANSWER 10 OF 68 MEDLINE
AN 72239121 MEDLINE
DN 72239121 PubMed ID: 4558236
TI Treatment of **aphthous** ulceration with **topical**

azathioprine. A double blind trial.
AU Eggleston D J; Nally F F
SO BRITISH JOURNAL OF ORAL SURGERY, **(1972 Mar)** 9 (3) 233-6.
Journal code: 0400651. ISSN: 0007-117X.
CY SCOTLAND: United Kingdom
DT (CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
(RANDOMIZED CONTROLLED TRIAL)
LA English
FS Dental Journals; Priority Journals
EM 197209
ED Entered STN: 19900310
Last Updated on STN: 19900310
Entered Medline: 19720921
TI Treatment of **aphthous** ulceration with **topical azathioprine**. A double blind trial.
SO BRITISH JOURNAL OF ORAL SURGERY, **(1972 Mar)** 9 (3) 233-6.
Journal code: 0400651. ISSN: 0007-117X.
CT Check Tags: Female; Human; Male
Adolescent
Adult
Autoimmune Diseases
Azathioprine: AD, administration & dosage
***Azathioprine: TU, therapeutic use**
Clinical Trials
Immunosuppression
Leukocytosis: ET, etiology
Middle Age
Placebos
***Stomatitis, Aphthous: DT, drug therapy**
Stomatitis, Aphthous: ET, etiology
RN **446-86-6 (Azathioprine)**

L9 ANSWER 11 OF 68 MEDLINE
AN 70130232 MEDLINE
DN 70130232 PubMed ID: 5417153
TI [Immunosuppressive therapy of **pemphigus vulgaris** and bullous **pemphigoid**].
Versuch einer immunsuppressiven Therapie von **Pemphigus vulgaris** und bullosen Pemphigoiden.
AU Herzberg J J
SO ARCHIV FUR KLINISCHE UND EXPERIMENTELLE DERMATOLOGIE, **(1970)** 237 (1) 76-8.
Journal code: 1256765. ISSN: 0300-8614.
CY GERMANY, WEST: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA German
FS Priority Journals
EM 197004
ED Entered STN: 19900101
Last Updated on STN: 19900101
Entered Medline: 19700419
TI [Immunosuppressive therapy of **pemphigus vulgaris** and bullous **pemphigoid**].
Versuch einer immunsuppressiven Therapie von **Pemphigus vulgaris** und bullosen Pemphigoiden.
SO ARCHIV FUR KLINISCHE UND EXPERIMENTELLE DERMATOLOGIE, **(1970)** 237 (1) 76-8.
Journal code: 1256765. ISSN: 0300-8614.
CT Check Tags: Female; Human; Male

Adult
Aged
***Azathioprine: TU, therapeutic use**
Drug Tolerance
Prednisolone, Topical: TU, therapeutic use
*Skin Diseases: DT, drug therapy
RN **446-86-6 (Azathioprine)**
CN 0 (Prednisolone, **Topical**)

L9 ANSWER 12 OF 68 USPATFULL
AB A pharmaceutical composition, which contains glycosaminoglycan having at least one sulfate group or a pharmaceutically acceptable salt thereof, and an immunosuppressant.
AN 2003:81725 USPATFULL
TI Anti-inflammatory agent
IN Kyogashima, Mamoru, Higashiyamato, JAPAN
Asari, Akira, Iruma, JAPAN
PA Seikagaku Corporation, Tokyo, JAPAN (non-U.S. corporation)
PI US 6537977 B1 20030325
WO 9711096 19970327 <--
AI US 1998-43124 19980512 (9)
WO 1996-JP2706 19960919
PRAI JP 1995-266409 19950919
DT Utility
FS GRANTED
EXNAM Primary Examiner: Wilson, James O.; Assistant Examiner: White, Everett
LREP Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 969
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6537977 B1 20030325
WO 9711096 19970327 <--
SUMM . . . focusing on these causes. For example, with respect to immunological anomalies, an immunosuppressant such as adrenocorticosteroid, cyclophosphamide, mizoribine, methotrexate or **azathioprine** is used in a usual manner or in salvage intravenous infusion therapy (pulse therapy). However, adrenocorticosteroid shows serious adverse effects. . .
SUMM . . . Di-OS (.DELTA.HexA1.fwdarw.3GalNAc); with 0.8-2.0 of intrinsic viscosity (100 mL/g); with 25,000-100,000, preferably 30,000-60,000, of molecular weight which was determined by **gel** permeation method using high performance liquid chromatography and glycosaminoglycan whose molecular weight was known as a standard (see reference example. . .

DETD . . . (2)with 0.8-2.0 of intrinsic viscosity (100 ml/g); (3) with 25,000-100,000, preferably 30,000-60,000, of average molecular weight which was determined by **gel** permeation method using high performance liquid chromatography and glycosaminoglycan whose molecular weight was known as a standard (the method described. . .
DETD . . . intramuscularly or percutaneously. Eyedrops thereof can be prepared in combination with an appropriate acceptable auxiliary ingredient and used for instillation. **Ointment** or **cream** thereof can be also prepared in combination with an appropriate base and coated on skin or mucosa. Further, oral drug. . .
DETD . . . "Today's remedy(1994)", 1994, Nankodo publ; 157-161p

"Immunosuppressants", and 162-183p, "Adrenocortical steroid"). As an immunosuppressant or an immunosuppressing compound, adrenocorticosteroid, cyclophosphamide, **azathioprine**, mizoribine, cyclosporine, methotrexate, tacrolimus hydrate, etc. can be exemplified. As typical adrenocorticosteroid, prednisolone, methyl prednisolone, betamethasone, dexamethasone, paramethasone, triamcinolone, hydrocortisone,. . .

DETD . . . idiopathic interstitial pneumonia, lung fibrosis and the like; renal diseases include interstitial nephritis and the like; dermal diseases include pemphigus, **pemphigoid**, immunogenic hydrosa acquired epidermolysis bullosa and the like; ophthalmic diseases include lens-induced uveitis, Vogt-Koyanagi-Harada syndrome and the like (Autoimmune diseases. . .

DETD . . . Using glycosaminoglycan with known molecular weight determined by light scattering method as standard, it was determined by eluting position of **gel** permeation using high performance liquid chromatography (HPLC), wherein three columns, that is, TSK **gel** G4000PWX.sub.L, TSK **gel** G3000PWX.sub.L and TSK **gel** G2500PWX.sub.L (anyone 300.times.7.8 mm in the inside diameter, Toso) which were connected with the next one, were used.

DETD . . . and injected into ampoules. Depending on the kind of immunosuppressant, the dose thereof was determined, that is, cyclophosphamide, 30 mg; **azathioprine**, 30 mg; mizoribine, 150 mg; methotrexate, 3 mg; tacrolimus hydrate, 3 mg; cyclosporine 15 mg.

DETD . . . of the above immunosuppressants, DS preparations can be administered. For example, 25 mg/day of prednisolone, 30 mg/day of cyclophosphamide or **azathioprine**, 150 mg/day of mizoribine, 3 mg/day of methotrexate or tacrolimus hydrate or 15 mg/day of cyclosporine can be used. ·

DETD . . . having sulfate group or pharmaceutically acceptable salt thereof is used in combination with an immunosuppressant such as adrenocortical steroid, cyclophosphamide, **azathioprine**, mizoribine, cyclosporine, methotrexate, tacrolimus hydrate etc., smaller amount of immunosuppressant than in the case of single administration of an immunosuppressant. . .

CLM What is claimed is:

. . . to unsaturated dissacharide and high performance liquid chromatography, and wherein said dermatan sulfate has an average molecular weight determined by **gel** permeation chromatography using a glycosaminoglycan of a known molecular weight as a standard and high performance liquid chromatography of from. . .

L9 ANSWER 13 OF 68 USPATFULL

AB The invention pertains to a process for the production of a pharmaceutical composition effective for controlling in a recipient mammalian host, particularly man, immune reactions of the type that are involved in graft of foreign tissue or cells, particularly transplantation of foreign tissues, organs or cells, particularly of allogeneic or even xenogeneic origin, or in immunodeficiency-linked diseases, which pharmaceutical composition is characterized by an active principle consisting of pooled transferrin-derived glycans obtained from a number of donors sufficient to allow the pool to contain sufficient phenotypic information required to ensure an induction of tolerance against antigens in an immuno-depressed host grafted with said antigens,

after that host had been administered an amount of such pooled transferrin glycans effective to induce said tolerance.

AN 2001:173143 USPATFULL
TI Transferrin glycans composition for the induction of immune tolerance
IN Pierpaoli, Walter, Belinzona, Switzerland
Kistler, Gonzague S., Uitikon Waldegg, Switzerland
PA Cellena AG, Ebmatingen, Switzerland (non-U.S. corporation)
PI US 6299878 B1 20011009
WO 9703680 19970206 <--
AI US 1998-983227 19980406 (8)
WO 1996-EP3159 19960718
19980406 PCT 371 date
19980406 PCT 102(e) date
PRAI EP 1995-401729 19950720
DT Utility
FS GRANTED
EXNAM Primary Examiner: Chan, Chrstina Y.; Assistant Examiner: VanderVegt, F. Pierre
LREP Birch, Stewart, Kolasch & Birch, LLP
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 1263
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 6299878 B1 20011009
WO 9703680 19970206 <--

SUMM . . . which are involved in the so-called host versus-graft reaction (HvGR) and so-called graft versus-host reaction (GvHR) or graft versus-host disease (**GvHD**), as well as immune reactions which are brought into play in bone marrow transplantation (BMT), i.e. when the host is. . .

SUMM . . . transplanted with bone marrow from BALB/c donors with iron-saturated human transferrin or conalbumin, resulted in remarkably stable engraftment, avoidance of **GvHD** and enduring chimerism in the majority of test animals (Pierpaoli W. et al, Cell Immunol 1991;134:225-234).

DETD . . . Netherlands). Antigen protein was determined by radial immunodiffusion with Nor-Partigen Tf and serum protein standard from Behring (Marburg, Germany). Agarose **gel** electrophoresis was performed with a Helena millipore (Milford, USA) 625 LC chromatograph; the conditions were the followings: column TSK3000SW (75. . .

DETD . . . or in combination were studied for their capacity to produce a temporary but deep depression of immunity as e.g., busulphan, **azathioprine**, cyclosporin, methotrexate, cyclophosphamide, prednisolone. At the end of our long-term trials, a combination of prednisolone acetate (Pr) and cyclophosphamide (Cy). . .

DETD . . . is non-toxic. After centrifugation (2000 rpm for 5 min) the supernatant fluid was removed from each tube leaving the cell **pellet** at the bottom and tubes were immersed into ice. Before reading, 25 .mu.l Trypan Blue solution (SIGMA) was added to. . .

DETD . . . the recipient. This procedure may change and/or improve the engraftment capacity of the donor bone marrow and enhance induction of **GvHD**-free chimerism.

L9 ANSWER 14 OF 68 USPATFULL
AB Human antibodies, preferably recombinant human antibodies, that specifically bind to human tumor necrosis factor .alpha.(hTNF.alpha.) are disclosed. These antibodies have high affinity for hTNF.alpha. (e.g., K.sub.d =10.sup.-8 M or less), a slow off rate for hTNF.alpha. dissociation (e.g., K.sub.off =10.sup.-3 sec.sup.-1 or less) and

neutralize hTNF.alpha. activity in vitro and in vivo. An antibody of the invention can be a full-length antibody or an antigen-binding portion thereof. The antibodies, or antibody portions, of the invention are useful for detecting hTNF.alpha. and for inhibiting hTNF.alpha. activity, e.g., in a human subject suffering from a disorder in which hTNF.alpha. activity is detrimental. Nucleic acids, vectors and host cells for expressing the recombinant human antibodies of the invention, and methods of synthesizing the recombinant human antibodies, are also encompassed by the invention.

AN 2001:107647 USPATFULL

TI Human antibodies that bind human TNF.alpha.

IN Salfeld, Jochen G., North Grafton, MA, United States
Allen, Deborah J., Cambridge, United Kingdom
Hoogenboom, Hendricus R. J. M., Hertogsingel, MA, United States
Kaymakcalan, Zehra, Westboro, MA, United States
Labkovsky, Boris, Framingham, MA, United States
Mankovich, John A., Andover, MA, United States
McGuinness, Brian T., Comberton, United Kingdom
Roberts, Andrew J., Cambridge, United Kingdom
Sakorafas, Paul, Newton, MA, United States
Schoenhaut, David, Garfield, NJ, United States
Vaughan, Tristan J., Impington, United Kingdom
White, Michael, Framingham, MA, United States
Wilton, Alison J., Cambridge, United Kingdom

PA BASF Aktiengesellschaft, Rheiland-Pfalz, Germany, Federal Republic of (non-U.S. corporation)

PI US 6258562 B1 20010710
WO 9729131 19970814 <--

AI US 1999-125098 19990316 (9)
WO 1997-US2219 19970210
19990316 PCT 371 date
19990316 PCT 102(e) date

RLI Continuation-in-part of Ser. No. US 1996-599226, filed on 9 Feb 1996, now patented, Pat. No. US 6090382

PRAI US 1996-31476P 19961125 (60)

DT Utility

FS GRANTED

EXNAM Primary Examiner: Saunders, David

LREP Lahive & Cockfield, LLP, DeConti, Jr., Giulio A., Hanley, Elizabeth A.

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 10 Drawing Figure(s); 11 Drawing Page(s)

LN.CNT 2754

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6258562 B1 20010710
WO 9729131 19970814 <--

DETD . . . (non-steroidal anti-inflammatory drug); Indomethacin (non-steroidal anti-inflammatory drug); Sulfasalazine (see e.g., Arthritis & Rheumatism (1996) Vol. 9, No. 9 (supplement), S281); **Azathioprine** (see e.g., Arthritis & Rheumatism (1996) Vol. 39 No. 9 (supplement), S281); ICE inhibitor (inhibitor of the enzyme interleukin-1.beta. converting. . .

DETD . . . or antibody portion, of the invention can be combined include the following: budenoside; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-**mercaptopurine**; **azathioprine**; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1.beta. monoclonal antibodies; anti-IL-6 monoclonal antibodies; growth factors;. . .

DETD . . . sclerosis with which an antibody, or antibody portion, of the invention can be combined include the following: corticosteroids; prednisolone; methylprednisolone; **azathioprine**; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon-.beta.1a (Avonex.TM.; Biogen); interferon-.beta.1b (Betaseron.TM.; Chiron/Berlex); Copolymer 1 (Cop1; Copaxone.TM.; Teva Pharmaceutical Industries, Inc.); hyperbaric. . .

DETD . . . antibody portion at a site of inflammation may be beneficial (e.g., local administration in the joints in rheumatoid arthritis or **topical** application to diabetic ulcers, alone or in combination with a cyclohexane-ylidene derivative as described in PCT Publication No. WO 93/19751).. . .

DETD Tumor necrosis factor has been implicated as a key mediator of allograft rejection and graft versus host disease (**GVHD**) and in mediating an adverse reaction that has been observed when the rat antibody OKT3, directed against the T cell. . . portions, of the invention, can be used to inhibit transplant rejection, including rejections of allografts and xenografts and to inhibit **GVHD**. Although the antibody or antibody portion may be used alone, more preferably it is used in combination with one or more other agents that inhibit the immune response against the allograft or inhibit **GVHD**. For example, in one embodiment, an antibody or antibody portion of the invention is used in combination with OKT3 to. . .

DETD . . . (i.e., unbound) .sup.125 I-labeled rhTNF.alpha. was removed by microcentrifugation for five minutes. Then, each test tube end containing a cell **pellet** was cut with the aid of a microtube scissor (Bel-Art 210180001, Bel-Art Products, Pequannock, N.J.). The cell **pellet** contains .sup.125 I-labeled rhTNF.alpha. bound to the p60 or p80 TNF.alpha. receptor, whereas the aqueous phase above the oil mixture contains excess free .sup.125 I-labeled rhTNF.alpha.. All cell **pellets** were collected in a counting tube (Falcon 2052, Becton Dickinson Labware, Lincoln Park, N.J.) and counted in a scintillation counter.

L9 ANSWER 15 OF 68 USPATFULL

AB An external preparation for **topical** administration which aims at inhibiting rejection reactions at organ or bone marrow transplantation or treating autoimmune diseases or allergic diseases and contains as the active ingredient 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol or a pharmaceutically acceptable acid addition salt thereof.

AN 2000:125105 USPATFULL

TI **Topical** administration of 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol

IN Fujii, Tsuneo, Fukuoka, Japan
Mishina, Tadashi, Fukuoka, Japan
Teshima, Koji, Saitama, Japan
Imayoshi, Tomonori, Fukuoka, Japan

PA Yoshitomi Pharmaceutical Industries, Ltd., Osaka-fu, Japan (non-U.S. corporation)

PI US 6121329 20000919
WO 9724112 19970710

AI US 1997-894728 19970827 (8)
WO 1996-JP3757 19961224
19970827 PCT 371 date
19970827 PCT 102(e) date

PRAI JP 1995-342503 19951228
DT Utility
FS Granted
EXNAM Primary Examiner: Page, Thurman K.; Assistant Examiner: Seidleck, Brian K.
LREP Birch, Stewart, Kolasch & Birch, LLP
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 659
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI **Topical** administration of 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol
PI US 6121329 20000919
WO 9724112 19970710
AB An external preparation for **topical** administration which aims at inhibiting rejection reactions at organ or bone marrow transplantation or treating autoimmune diseases or allergic diseases.
.
.
SUMM This invention relates to an external preparation 2-Amino-2-(2-(4-Octylphenyl)Ethyl)Propane-1,3-Diol Or Pharmaceutically Acceptable Salts Thereof For **Topical** Administration for **topical** administration, in more detail to an external preparation for **topical** administration which contains as the active ingredient 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol or pharmaceutically acceptable acid-addition salts thereof.
SUMM Namely, the present invention relates to an external preparation for **topical** administration which contains as the active ingredient 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol or pharmaceutically acceptable acid-addition salts thereof (hereunder sometimes referred to as the. . .
SUMM The external preparation for **topical** administration which is applicable to the compound of the present invention includes an **ointment**, a paste, a liniment, a lotion, a plaster, a cataplasm, an eye drop, an eye **ointment**, a suppository, a fomentation, an inhalant, a spray, an aerosol, a paint, a nasal drop, a **cream**, a tape, a patch and the like.
SUMM The external preparation for **topical** administration of the present invention contains the compound of the present invention in a form of a mixture with an. . .
SUMM . . . mixed with, for example, a non-toxic and pharmaceutically acceptable carrier which is usually employed for obtaining an external preparation for **topical** administration.
SUMM The compound of the present invention as an active ingredient of the external preparation for **topical** administration of the present invention can be contained in an amount enough to exhibit the desired activity depending on the. . . induced from immune disorder as mentioned below, the compound of the present invention can be administered by way of a **topical** administration, an aerosol or a rectal administration in a form of a dosage unit composition which contains pharmaceutically acceptable and. . .
SUMM The external preparation for **topical** administration containing the compound of the present invention will be explained in more detail as follows:
SUMM When the compound of the present invention is used in the form of an **ointment**, it is contained in an amount of 0.01 to 10 w/w % in the **ointment**.
SUMM The **ointment** base which can be used includes oleaginous base (a natural wax such as white beeswax or carnauba wax, a petroleum. .
.

paraffin, white soft paraffin or yellow petrolatum, plastibase, zelen 50W, silicone, a vegetable oil, pork tallow, beef tallow, a simple **ointment** or lead oleate plaster), an emulsion type **ointment** base (an O/W type base such as a hydrophilic **ointment** or a vanishing **cream** or a W/O type base such as a hydrophilic petrolatum, a purified lanolin, aquahole, eucelin, neocelin, an absorptive **ointment**, a hydrated lanolin, cold **cream**, a hydrophilic plastibase), a water-soluble base (a macrogol **ointment** or solbase) or a suspension type **ointment** base (a lyogel base, i.e. a hydrogel base such as a non-fat **ointment**, a gelbase or lotion; or an FAPG base (a suspension of a microparticle of an aliphatic alcohol such as stearyl alcohol or cetyl alcohol in propylene glycol), and these **ointment** base can be used alone or in a combination of not less than two bases.

SUMM Further, when to be used as an **ointment**, the compound of the present invention is dissolved in a solubilizing and absoptive accelerating agent and added to the above-mentioned **ointment** base.

SUMM . . . % and which can accelerate the absorption of the compound of the present invention from skin when formulated as an **ointment**, and includes a lower alkanediol (e.g. ethylene glycol, propylene glycol or butylene glycol), an alkylene carbonate (e.g. propylene carbonate or. . . of the compound of the present invention. The upper amount is limited not to deteriorate the physicochemical properties of the **ointment**.

SUMM The **ointment** which contains the compound of the present invention may contain, in addition to the above-mentioned **ointment** base, other additives such as an emulsifier (e.g. polyoxyethylene hardened caster oil, glycerol monostearate, sorbitan sesquioleate or lauromacrogol); a suspending. . .

SUMM The **ointment** of the present invention can be prepared by mixing a solution containing the compound of the present invention with an **ointment** base in accordance with a conventional method. In the process of formulation, not less than one of the adjuvant or additive mentioned above can be simultaneously added to the **ointment** base. Furthermore, the **ointment** can be manufactured by dissolving the compound of the present go invention in the solubilizing and absoptive accelerating agent, admixing the obtained solution with the **ointment** base, stirring the obtained mixture under heating, and then cooling the resultant mixture.

SUMM The **ointment** containing the compound of the present invention can be used by applying to the affected part of the skin once. . .

SUMM . . . present invention can be prepared by using the same base and according to the same method as those of the **ointment** as mentioned above.

SUMM . . . forms such as a rectal suppository which is solid at the normal temperature and melts at a body temperature; an **ointment** or liquid enema which can be prepared by dissolving or suspending the compound of the present invention in a liquid. . .

SUMM The external preparation for **topical** administration of the present invention can be used for the prevention or treatment of various medical indications, which have been. . . limb, muscle, nervus, fatty marrow, duodenum, skin or pancreatic islet cell etc., including xeno-transplantation), graft-versus-host diseases by bone marrow

transplantation (**GvHD**), autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, nephrotic syndrome lupus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type I. . .

SUMM . . . as psoriasis, psoriatic arthritis, atopic eczema (atopic dermatitis), contact dermatitis and further eczematous dermatitises, seborrheic dermatitis, lichen planus, pemphigus, bullous **pemphigoid**, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne, alopecia areata, eosinophilic fasciitis, and atherosclerosis.

SUMM . . . (prednisolone, methylprednisolone, dexamethasone, hydrocortisone and the like) or nonsteroidal anti-inflammatory agent. As the other immunosuppressant, preferred is particularly selected from **azathioprine**, brequinar sodium, deoxyspergualin, mizoribine, mycophenolate 2-morphorinoethyl, cyclosporin, rapamycin, tacrolimus monohydrate.

DETD . . . was dissolved in 19 g of hydrophilic petrolatum under heating at 60.degree. C., and cooled with stirring to prepare an **ointment** containing 5% of Compound (I).

DETD . . . was mixed well with 19 g of plastibase (gelled hydrocarbon) in a mortar for about 30 minutes to prepare an **ointment** containing 5% of Compound (I).

DETD The external preparation containing the compound of the present invention is a useful **topical** preparation for inhibiting the rejection reactions at organ or bone marrow transplantation or treating the autoimmune diseases or allergic diseases.

CLM What is claimed is:

. . . effective amount of a composition of 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol or a pharmaceutically acceptable acid addition salt thereof to said subject by a **topical** or ocular administration route.

. . . effective amount of a composition of 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol or a pharmaceutically acceptable acid addition salt thereof to said subject by a **topical** or ocular administration route.

. . . effective amount of a composition of 2-amino-2-(2-(4-octylphenyl)ethyl)propane-1,3-diol or a pharmaceutically acceptable acid addition salt thereof to said subject by a **topical** or ocular administration route.

. . . 1 or 2, wherein said disease or disorder is induced from immune disorder, wherein said composition is administered via a **topical** administration route.

7. The method according to claim 1, wherein the other immunosuppressant is **azathioprine**, brequinar sodium, deoxyspergualin, mizoribine, mycophenolate 2-morpholinoethyl, cyclosporin, rapamycin, or tacrolimus monohydrate.

13. The method according to claim 6, wherein the other immunosuppressant is **azathioprine**, brequinar sodium, deoxyspergualin, mizoribine, mycophenolate 2-morpholinoethyl, cyclosporin, rapamycin, or

tacrolimus monohydrate.

14. The method according to claim 9, wherein the other immunosuppressant is **azathioprine**, brequinar sodium, deoxyspergualin, mizoribine, mycophenolate 2-morpholinoethyl, cyclosporin, rapamycin, or tacrolimus monohydrate.

15. The method according to claim 10, wherein the other immunosuppressant is **azathioprine**, brequinar sodium, deoxyspergualin, mizoribine, mycophenolate 2-morpholinoethyl, cyclosporin, rapamycin, or tacrolimus monohydrate.

16. The method according to claim 11, wherein the other immunosuppressant is **azathioprine**, brequinar sodium, deoxyspergualin, mizoribine, mycophenolate 2-morpholinoethyl, cyclosporin, rapamycin, or tacrolimus monohydrate.

17. The method according to claim 12, wherein the other immunosuppressant is **azathioprine**, brequinar sodium, deoxyspergualin, mizoribine, mycophenolate 2-morpholinoethyl, cyclosporin, rapamycin, or tacrolimus monohydrate.

L9 ANSWER 16 OF 68 USPATFULL

AB The invention concerns pharmaceutically useful peptide derivatives of the formula (I): P--R.sup.1 --R.sup.2 --R.sup.3 --R.sup.4, in which P, R.sup.1, R.sup.2, R.sup.3, and R.sup.4 have the various meanings defined herein, and their pharmaceutically acceptable salts, and pharmaceutical compositions containing them. The novel peptide derivatives are of value in treating MHC class II dependent T-cell mediated autoimmune or inflammatory diseases, such as rheumatoid arthritis. The invention further concerns processes for the manufacture of the novel peptide derivatives and the use of the compounds in medical treatment.

AN 2000:88165 USPATFULL

TI Peptide derivatives useful in treating autoimmune diseases

IN Edwards, Philip Neil, Macclesfield, United Kingdom
Luke, Richard William Arthur, Macclesfield, United Kingdom
Cotton, Ronald, Macclesfield, United Kingdom

PA Zeneca Limited, Macclesfield, United Kingdom (non-U.S. corporation)

PI US 6087336 20000711
WO 9731023 19970828 <--

AI US 1998-125517 19980820 (9)
WO 1997-GB438 19970218
19980820 PCT 371 date
19980820 PCT 102(e) date

PRAI GB 1996-3855 19960223
GB 1996-20819 19961005

DT Utility

FS Granted

EXNAM Primary Examiner: Russel, Jeffrey E.

LREP Rothwell, Figg, Ernst & Kurz

CLMN Number of Claims: 15

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 2999

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6087336 20000711

WO 9731023 19970828 <--

SUMM . . . ankylosing spondylitis, Sjogren syndrome, myasthenia gravis; Type I (insulin dependent) diabetes, Hashimoto's disease, Grave's disease, Addison's disease, scleroderma, polymyositis, dermatomyositis, **pemphigus vulgaris**, bullous **pemphigoid** autoimmune haemolytic anaemia, pernicious anaemia, glomerulonephritis, graft rejections and such like, especially rheumatoid arthritis and multiple sclerosis.

SUMM . . . infusion), for example a sterile aqueous or oily solution or suspension. The composition may be in a form suitable for **topical** administration such as for example **creams**, **ointments** and **gels**. Skin patches are also contemplated. Formulation in general is described in Chapter 25.2 of Comprehensive Medicinal Chemistry, Volume 5, Editor. . .

SUMM . . . NSAID (such as ibuprofen or piroxicam), an analgesic (such as paracetamol), a corticosteroid, a muscle relaxant, a lipoxygenase inhibitor, methotrexate, **azathioprine**, D-penicillamine, Cyclosporin A or a monoclonal antibody therapy (such as anti-CD4 or anti-TNF). In diabetes the peptide derivative may be. . .

DETD . . . material was removed by evaporation to give 4-(2-guanidinoethyl)aniline dihydrochloride as a brown foam (0.984 g) (after drying over potassium hydroxide **pellets**).

L9 ANSWER 17 OF 68 USPATFULL

AB Compounds and methods for use in immunosuppressive and anti-inflammatory treatment, and for inhibiting male fertility, are described. The compounds are triptolide analogs with improved water solubility and low toxicity.

AN 1999:121418 USPATFULL

TI Immunosuppressive compounds and methods

IN Qi, You Mao, Los Altos, CA, United States
Musser, John H., San Carlos, CA, United States
Fidler, John M., Oakland, CA, United States

PA Pharmagenesis, Inc., Palo Alto, CA, United States (U.S. corporation)

PI US 5962516 19991005
WO 9731921 19970904 <--

AI US 1999-142128 19990125 (9)
WO 1997-US3202 19970228
19990125 PCT 371 date
19990125 PCT 102(e) date

DT Utility

FS Granted

EXNAM Primary Examiner: Reamer, James H.

LREP Gorthey, LeeAnn, Powers, Vincent M.

CLMN Number of Claims: 11

ECL Exemplary Claim: 1,4

DRWN 9 Drawing Figure(s); 8 Drawing Page(s)

LN.CNT 1309

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5962516 19991005
WO 9731921 19970904 <--

SUMM . . . drugs and low dose corticosteroids); disease-modifying antirheumatic drugs, known as "DMARDs" (antimalarials, gold salts, penicillamine, and sulfasalazine) and immunosuppressive agents (**azathioprine**, chlorambucil, high dose corticosteroids, cyclophosphamide, methotrexate, nitrogen mustard, 6-**mercaptopurine**, vincristine, hydroxyurea, and cyclosporin A). None of the available drugs are completely effective, and most are limited by severe toxicity.

SUMM Another obstacle in transplantation, which has limited bone marrow transplants (BMT) in particular, is graft-versus-host disease (**GVHD**). **GVHD** is a condition in which transplanted marrow cells attack the recipient's cells (Thomas, 1975; Storb, 1984). Many BMT patients receiving HLA-identical marrow that tests negative in the mixed lymphocyte reaction (MLR) still develop **GVHD**, presumably because of a disparity between the recipient and donor at polymorphic non-HLA determinants. A large proportion of **GVHD** -afflicted individuals die as a result of **GVHD** (Weiden et al., 1980).

SUMM . . . for preventing transplant rejection include corticosteroids, antimetabolite drugs that reduce lymphocyte proliferation by inhibiting DNA and RNA synthesis such as **azathioprine**, immunosuppressive drugs such as cyclosporin A, which specifically inhibits T cell activation, and specific antibodies directed against T lymphocytes or. . .

DETD . . . powder, or liquid dosage forms, such as, for example, tablets, pills, capsules, powders, sustained-release formulations, solutions, suspensions,emulsions, suppositories, retention enemas, **creams** , **ointments**, lotions, aerosols or the like, preferably in unit dosage forms suitable for simple administration of precise dosages.

DETD . . . of the present invention may be employed in immunosuppression therapy, in particular, therapy in treating an autoimmune disease, graft-versus-host disease (**GVHD**), or transplantation rejection, particularly allograft rejection or xenograft rejection. The compositions are also useful for inhibiting male fertility, for treatment. . .

DETD . . . heart, kidney, liver, cellular, and bone marrow transplants. The method may also be used in the treatment of graft-versus-host disease (**GVHD**), in which transplanted immune cells attack the allogeneic host. Initial treatment is administered perioperatively. In addition, the composition may be. . .

DETD . . . concurrently with another immunosuppressive drug. The method includes administering to the subject, an immunosuppressant drug such as

cyclosporin A, FK506, **azathioprine**, rapamycin, mycophenolic acid, or a glucocorticoid, in an amount that is substantially less than the dose needed to achieve effective. . . analog of formula 1, as described above, is administered in an amount effective to suppress allograft rejection, xenograft rejection, or **GVHD** in the host, when administered in combination with the immunosuppressive compound. By

"an amount that is substantially less than the dose needed to achieve effective suppression of allograft rejection (or xenograft rejection, or

rejection due to **GVHD**) when the compound is administered alone" is meant an amount of immunosuppressant drug which is below 50%, and preferably less. . .

DETD (c) **azathioprine**, or 6-[(1-methyl-4-nitro-1H-immidazole-5yl)thio]1H-purine;

DETD . . . unable to swallow, or oral absorption is otherwise impaired, the preferred systemic route of administration will be parenteral, intranasal, or **topical**.

DETD . . . is worked up by filtering off the dicyclohexylurea, removing the solvent by evaporation, and chromatographing the obtained solid on silica **gel**.

DETD . . . The dicyclohexylurea is filtered off, and the solvent is removed by evaporation. The crude product is then chromatographed on silica **gel**.

DETD . . . is worked up by filtering off the dicyclohexylurea, removing

the solvent by evaporation, and chromatographing the obtained solid on silica **gel**.

L9 ANSWER 18 OF 68 USPATFULL
AB Methods are described for identifying the amino acid residues of an antibody variable domain which may be modified without diminishing the native affinity of the domain for antigen while reducing its immunogenicity with respect to a heterologous species and for preparing so modified antibody variable domains which are useful for administration to heterologous species. Antibody variable regions prepared by the methods of the invention are also described.
AN 1998:68811 USPATFULL
TI Modified antibody variable domains
IN Studnicka, Gary M., Santa Monica, CA, United States
Little, II, Roger G., Benicia, CA, United States
Fishwild, Dianne M., Hayward, CA, United States
Kohn, Fred R., Walnut Creek, CA, United States
PA Xoma Corporation, Berkeley, CA, United States (U.S. corporation)
PI US 5766886 19980616 <--
WO 9311794 19930624 <--
AI US 1993-107669 19930813 (8)
WO 1992-US10906 19921214
19930813 PCT 371 date
19930813 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1991-808464, filed on 13 Dec 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Feisee, Lila; Assistant Examiner: Lucas, John
LREP McAndrews, Held & Malloy, Ltd.
CLMN Number of Claims: 23
ECL Exemplary Claim: 1,10,17
DRWN 26 Drawing Figure(s); 24 Drawing Page(s)
LN.CNT 2865
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5766886 19980616 <--
WO 9311794 19930624 <--
DETD . . . lupus erythematosus and lupus nephritis), scleroderma diseases (including lichen sclerosis, morphea and lichen planus), rheumatoid arthritis and the spondylarthropathies, thyroiditis, **pemphigus vulgaris**, diabetes mellitus type 1, progressive systemic sclerosis, aplastic anemia, myasthenia gravis, myositis including polymyositis and dermatomyositis, Sjogren's disease, collagen vascular. . .
DETD . . . agents useful in suppressing allergic or other undesired reactions of a host. Immunosuppressive agents include prednisone, prednisolone, dexamethasone, cyclophosphamide, cyclosporine, 6-**mercaptopurine**, methotrexate, **azathioprine**, and gamma globulin. All of these agents are administered in generally accepted efficacious dose ranges such as those disclosed in. . .
DETD . . . preferred embodiment of the present invention, anti-pan T cell immunoglobulins may be formulated into various preparations such as injectable and **topical** forms. Parenteral formulations are preferred for use in the invention, most preferred is intramuscular (i.m.) or intravenous (i.v.) administration. The. . .
DETD Alternatively, anti-pan T cell immunoglobulin is formulated into **topical** preparations for local therapy by including a therapeutically effective concentration of anti-pan T cell immunoglobulin in a dermatological vehicle. **Topical** preparations may be useful to treat skin lesions such as psoriasis and

dermatitis associated with lupus. The amount of anti-pan T cell immunoglobulin to be administered, and the anti-pan T cell immunoglobulin concentration in the **topical** formulations, will depend upon the vehicle selected, the clinical condition of the patient,
the systemic toxicity and the stability of. . .

DETD The concentration of anti-pan T cell immunoglobulin for **topical** formulations is in the range from about 0.1 mg/ml to about 25 mg/ml. Typically, the concentration of anti-pan T cell immunoglobulin for **topical** formulations is in the range from about 1 mg/ml to about 20 mg/ml. Solid dispersions of anti-pan T cell immunoglobulin. . . vehicle may be useful with 1% w/w hydrogel vehicles in the treatment of skin inflammation. Suitable vehicles, in addition to **gels**, are oil-in-water or water-in-oil emulsions using mineral oils, petrolatum, and the like.

DETD . . . be optionally administered topically by the use of a transdermal therapeutic system (Barry, Dermatological Formulations, p. 181 (1983)). While such **topical** delivery systems have been designed largely for transdermal administration of low molecular weight drugs, by definition they are capable of. . .

DETD . . . delivery may be employed and may contain excipients as described above for parenteral administration and other excipients used in a **topical** preparation such as cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Any pharmacologically acceptable buffer may be used, e.g.,. . .

DETD . . . (SEQ ID Nos. 42 and 43, respectively). Oligonucleotides greater
than 50 bp in length were purified on a 15% polyacrylamide **gel** in the presence of 25% urea. DNA strand extension and DNA amplification was accomplished with a Taq polymerase and the. . . in SEQ ID NO: 46.
The assembled V/J-region was cut with SalI and BstEII, purified by electrophoresis on an agarose **gel**, and assembled into a heavy chain expression vector, pING4612, which is similar to that described for heavy chain expression in. . .

DETD . . . in SEQ ID NO. 47. The assembled V/J-region was cut with SalI and HindIII, purified by electrophoresis on an agarose **gel**, and assembled into a light chain antibody expression vector, pING4614 similar to those described for light chain expression in Robinson. . .

DETD . . . was allowed to proceed for 45 minutes at 23.degree. C. .sup.125
I-cH65 IgG was purified from unbound .sup.125 I by **gel** filtration using a Sephadex G-25-80 column. Concentration and specific activity was determined by measuring the TCA-precipitated counts before and after. . .

DETD . . . C. At the end of 5 hours, binding was terminated by three washes with ice cold BHD using centrifugation to **pellet** cells. Radioactivity was determined by solubilizing bound .sup.125 I-cH65 IgG with 1N NaOH and counting in a Beckman Gamma 8000. . .

DETD . . . treated with T4 polymerase and then digested with AccI. The 274
base pair (bp) fragment was purified on an agarose **gel** and ligated along with the 141 bp SalI to AccI fragment from pING4619 into pUC18 cut with SalI and SmaI. . .

DETD . . . cycles as outlined above. The assembled V/J region was cut with
SalI and HindIII, purified by electrophoresis on an agarose **gel**, and assembled into a light chain antibody expression vector, pING4630.

DETD . . . of 20 .mu.l of 105 mM sodium metabisulfite and 120 mM potassium
iodine followed by centrifugation for 1 minute to **pellet** the beads. .sup.125 I-cH65 IgG was purified by **gel** filtration using 7 mls of sephadex G25, using PBS (137 mM NaCl, 1.47 mM KH.sub.2 PO.sub.4, 8.1 mM Na.sub.2 HPO.sub.4,. . .

L9 ANSWER 19 OF 68 USPATFULL
AB A method for the treatment of a cutaneous, ocular, or mucosal pathological condition which is associated with immune response in a human or other mammal, that includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.
AN 97:123224 USPATFULL
TI **Topical** application of spiperone or derivatives thereof for treatment of pathological conditions associated with immune responses
IN Sharpe, Richard J., Newtonville, MA, United States
Arndt, Kenneth A., Newton Centre, MA, United States
Galli, Stephen J., Winchester, MA, United States
Meltzer, Peter C., Lexington, MA, United States
Razdan, Raj K., Belmont, MA, United States
Sard, Howard P., Arlington, MA, United States
PA Beth Israel Deaconess Medical Center, Inc., Boston, MA, United States (U.S. corporation)
PI US 5703088 19971230 <--
AI US 1992-893536 19920604 (7)
RLI Continuation-in-part of Ser. No. US 1992-831429, filed on 5 Feb 1992, now patented, Pat. No. US 5244902 And a continuation-in-part of Ser. No.
US 1990-494744, filed on 16 Mar 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-396523, filed on 21 Aug 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Gerstl, Robert
LREP Kilpatrick Stockton LLP
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 1178
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI **Topical** application of spiperone or derivatives thereof for treatment of pathological conditions associated with immune responses
PI US 5703088 19971230 <--
AB . . . cutaneous, ocular, or mucosal pathological condition which is associated with immune response in a human or other mammal, that includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.
SUMM This invention is in the area of the **topical** treatment of cutaneous, ocular, and mucosal hypersensitivity and hyperproliferative conditions induced by or associated with an immune response, that includes. . .
SUMM . . . Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due
to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, lichen

planus, asthma, allergic asthma, cutaneous lupus erythematosus, dry eye associated with Sjogren's Syndrome,. . .

SUMM . . . agents with partial utility for treating some of the above conditions include psoralen plus ultraviolet A (PUVA), cyclosporin A, or **azathioprine**, but the risk-to-benefit ratios for these agents is unfavorable for most of the conditions described above.

SUMM U.S. Pat. No. 4,874,766 assigned to Janssen Pharmaceutica N.V. discloses a method for promoting wound-healing by **topical** administration of a serotonin-antagonist compound, including spiperone and its derivatives. Wound healing is a reparative process by which several types. . .

SUMM It is an object of the present invention to present a method for the **topical** treatment of cutaneous, mucosal and ocular pathology associated with immune responses.

SUMM It is yet another object of the present invention to present a method for the **topical** treatment of cutaneous, mucosal, or ocular hypersensitivity and epithelial hyperproliferation.

SUMM It is yet another object of the invention to present a method for the **topical** treatment of cutaneous, mucosal or ocular scarring.

SUMM . . . ocular, or mucosal condition in a human or other mammal resulting from pathology associated with an immune response, that includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.

SUMM . . . exhibits a strong immunosuppressive activity when applied topically. The parent spiperone is used herein as the model of an active **topical** immunosuppressant. Spiperone derivatives are measured against this model, and are considered to be immunosuppressants if they suppress the leukocyte infiltration. . .

SUMM . . . administered topically in a suitable carrier to effectively immunosuppress the patient at the site of application. Because the application is **topical**, i.e., local, immunosuppression is achieved without producing systemic effects, most notably, the significant neuroleptic effect that is associated with the. . .

SUMM Spiperone and its active derivatives are useful as **topical** agents in treating contact dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions. The novel. . .

DRWD . . . contact hypersensitivity reactions. These data (mean.+-.SEM) are from the same mice whose ear thickness measurements are presented in FIG. 5. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**p<0.01).

DRWD FIGS. 8a,b,c--Effect of **topical** treatment with spiperone on leukocyte infiltration associated with oxazolone-induced contact hypersensitivity reactions. These data (mean.+-.SEM) are from the same mice. . . are presented in FIGS. 7a,b,c. Biopsies were performed 24 hours (a, b) or 46 hours (c) after application of oxazolone. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**=p<0.01).

In

FIG. 8a, the slight. . .

DRWD FIG. 10--Effect of **topical** treatment with spiperone on leukocyte infiltration associated with DNFB-induced contact hypersensitivity reactions. These data (mean.+-.SEM) are from the same mice whose ear thickness measurements are presented in FIG. 9. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**p<0.01). The slight effect of treatment. . .

DETD Mammals, and specifically humans, suffering from pathogenic cutaneous, ocular, or mucosal immune responses can be treated by **topical** administration to the patient of an effective amount of spiperone, or its derivative or salt thereof, in the presence of. . .

DETD Solutions or suspensions for **topical** application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,. . .

DETD Suitable vehicles or carriers for **topical** application are known, and include lotions, suspensions, **ointments**, **creams**, **gels**, tinctures, sprays, powders, pastes, slow-release transdermal patches, aerosols for asthma, suppositories for application to rectal, vaginal, nasal or oral mucosa,. . .

DETD Thickening agents, emollients, and stabilizers can be used to prepare **topical** compositions. Examples of thickening agents include petrolatum, beeswax, xanthan gum, or polyethylene glycol, humectants such as sorbitol, emollients such as mineral oil, lanolin and its derivatives, or squalene. A number of solutions and **ointments** are commercially available, especially for ophthalmic and dermatologic applications.

DETD Natural or artificial flavorings or sweeteners can be added to enhance the taste of **topical** preparations applied for local effect to mucosal surfaces. Inert dyes or colors can be added, particularly in the case of. . .

DETD . . . potential irritancy or neuropharmacological effects of the composition. See, in general, Arndt, K. A., P. V. Mendenhall, "The Pharmacology of **Topical** Therapy", Dermatology in General Medicine, 1987; T. B. Fitzpatrick, A. Z. Eisen, K. Wolff, I. M. Freedberg and K. F.. . .

DETD Spiperone and spiperone derivatives are capable of suppressing the immune response in humans and other mammals on **topical** application. As such, the compounds, or therapeutic compositions thereof, are useful for the treatment of a myriad of immunological disorders. Pathogenic immune responses that can be treated by **topical** application of spiperone or spiperone derivatives include contact dermatitis, atopic dermatitis, eczematous dermatitis, drug eruptions, lichen planus, psoriasis, alopecia areata,. . . Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, cutaneous lupus erythematosus, scleroderma, allergic reactions secondary to arthropod bite reactions, **aphthous** ulcers, conjunctivitis, keratoconjunctivitis, iritis, asthma and allergic asthma, vaginitis, Crohn's disease, ulcerative colitis and proctitis. These compounds can also be. . .

DETD . . . ensues from the dry eye state. Spiperone or its active derivatives can be provided as an ophthalmic drop or ophthalmic **ointment** to humans or other mammals, including dogs and cats, in an effective amount in a suitable vehicle. This **topical** ophthalmic treatment can also serve to correct corneal and conjunctival

disorders exacerbated by tear deficiency and KCS, such as corneal. . .

DETD . . . the tissue swelling and the leukocyte infiltration associated with the elicitation phase of contact hypersensitivity to either oxazolone or dinitrofluorobenzene. **Topical** treatment with spiperone also suppressed the sensitization phase of contact sensitivity. However, mice treated topically with spiperone, unlike those treated. . .

DETD **Topical** Spiperone Treatment

DETD . . . infiltration at sites of hapten challenge than did vehicle-treated mice ($p<0.01$ for either comparison). These data show that treatment with **topical** spiperone can effectively inhibit the sensitization phase of cutaneous contact hypersensitivity.

DETD Effects of **Topical** Spiperone on Expression of Contact Hypersensitivity

DETD . . . skin) to both surfaces of the ears. The right ears of control mice were similarly treated, but with vehicle alone. **Topical** administration of a 4.0% suspension of spiperone in absolute ethanol, propylene glycol, and olive oil one hour after hapten challenge. . .

DETD Although **topical** application of spiperone was extremely effective in diminishing both the tissue swelling and the leukocyte infiltration associated with contact hypersensitivity. . .

DETD To evaluate the effect of **topical** treatment with spiperone on contact hypersensitivity reactions elicited with a different hapten, the effect of **topical** treatment with a 0.5% suspension of spiperone on the contact hypersensitivity reactions elicited with DNFB was examined. **Topical** treatment with spiperone significantly diminished the tissue swelling associated with reactions to DNFB (by 45%, FIG. 9) and had an. . .

DETD Mice were sensitized to oxazolone as described in Example 1. Three days later, slow release indomethacin **pellets** (0.05 mg, 3 week release) were implanted subcutaneously under light ether anesthesia. The dose of indomethacin delivered by these **pellets** has been previously shown to completely block prostaglandin synthesis in mice, by Jun, D. D., et al., J. Invest. Dermatol.. . .

DETD . . . and variations of the present invention relating to methods for the treatment of pathology associated with immune responses that includes **topical** administration of an effective amount of spiperone or a spiperone derivative will be obvious to those skilled in the art. . .

CLM What is claimed is:

. . . treatment of a cutaneous, ocular, or mucosal pathology associated with an immune response in a human or other mammal comprises **topical** application of an effective amount of a compound selected from the group consisting of a quaternary salt of spiperone and. . .

. . . alopecia areata, cutaneous lupus erythematosus, scleroderma, asthma, allergic asthma, ulcerative colitis, Crohn's disease, allergic reactions secondary to arthropod bite reactions, **aphthous** ulcers, conjunctivitis, iritis, keratoconjunctivitis, vaginitis, and proctitis.

L9 ANSWER 20 OF 68 USPATFULL

AB The compounds of Formula I ##STR1## are useful as immunosuppressive

agents.
AN 97:115314 USPATFULL
TI Triterpene derivatives with immunosuppressant activity
IN Baker, Robert K., Cranford, NJ, United States
Kayser, Frank, Hoboken, NJ, United States
Bao, Jianming, Westfield, NJ, United States
Parsons, William H., Belle Mead, NJ, United States
Rupprecht, Kathleen M., Cranford, NJ, United States
PA Merck & Co. Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5696156 19971209 <--
AI US 1996-733037 19961016 (8)
PRAI US 1995-8169P 19951031 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Dentz, Bernard
LREP Camara, Valerie J., Daniel, Mark R.
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2703
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5696156 19971209 <--
SUMM . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, Graves ophthalmopathy and asthma.
SUMM . . . pathogenic microorganisms, inflammatory and hyperproliferative skin diseases, psoriasis, atopical dermatitis, contact dermatitis, eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne, Alopecia areata, keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's. . .
SUMM . . . as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise). . .
SUMM . . . as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise). . .
SUMM . . . or more immunosuppressant agents. These immunosuppressant agents within the scope of this invention include, but are not limited to, IMUREK.RTM. **azathioprine** sodium, brequinar sodium, SPANIDIN.RTM. gusperimus trihydrochloride (also known as deoxyspergualin), mizoribine (also known as bredinin), CELLCEPT.RTM. mycophenolate mofetil, NEORAL.RTM.. Cyclosporin. . .
SUMM . . . ingredient compound with the site of action in the body of a warm-blooded animal. For example, administration, can be oral, **topical**, including transdermal, ocular, buccal, intranasal, inhalation, intravaginal, rectal, intracisternal and parenteral. The term "parenteral" as used herein refers to modes. . .
SUMM . . . as dispersions, suspensions or solutions. Other dosages forms

that can also be used to administer the active ingredient as an **ointment**, **cream**, drops, transdermal patch or powder for **topical** administration, as an ophthalmic solution or suspension formation, i.e., eye drops, for ocular administration, as an aerosol spray or powder composition for inhalation or intranasal administration, or as a **cream**, **ointment**, spray or suppository for rectal or vaginal administration.

DETD . . . This was first fractionated by preparative thin layer chromatography (TLC) on a 20 cm by 20 cm E. Merck silica **gel** 60F.sub.254 plate of 1 mm thickness using methylene chloride-ethyl acetate 1:1 (v/v) as solvent, then by high performance liquid chromatography. . .

DETD Homogeneity of the preparations was ascertained in several TLC systems, such as E. Merck silica **gel** 60F.sub.254, methylene chloride-ethyl acetate 1:1, Rf 1(a) 0.4, Rf 1(b) 0.3; Whatman KC.sub.18, methanol-water 9:1, Rf 1(a) 0.65, Rf 1(b). . .

DETD Partial purification of the methylene chloride extract was achieved by column chromatography on E. Merck silica **gel** 60 (120 ml), eluting with a step gradient of ethyl acetate in methylene chloride. The step gradient was designed so. . . afforded 100 mg and 20 mg respectively of 1(a) and 1(b) after crystallization from methanol. Later-eluting fractions from the silica **gel** column above were found to contain at least two related compounds based on UV spectra and color reactions on TLC. . .

DETD . . . chloride each time. The pooled methylene chloride extracts are evaporated down and fractionation proceeds by repeated column chromatography on silica **gel**. One employs methylene chloride-methanol 97:3 in a first step; the mixed compounds of Formula 1(a) and 1(b) thus obtained are resolved by chromatographing on fresh silica **gel** eluted with methylene chloride-ethyl acetate 3:1. Volume of elution for the compound of Formula 1(a) ranges from about 2 to. . .

DETD . . . chloride each time. The pooled methylene chloride extracts are evaporated down and fractionation proceeds by repeated column chromatography on silica **gel**. One employs methylene chloride-methanol 97:3 in a first step; the mixed compounds of Formula 1(a) and 1(b) thus obtained are resolved by chromatographing on fresh silica **gel** eluted with methylene chloride-ethyl acetate 3:1. Volume of elution for the compound of Formula 1(a) ranges from about 2 to. . .

DETD . . . was dissolved in a small amount of ethyl acetate/hexanes (2:1) (ca. 1 mL) and filtered through 30 g of silica **gel** eluting with 500 ml of ethyl acetate/hexanes (2:1). The first fractions, containing the Wilkinson-catalyst (approx. 50 mL) were discarried. The. . .

DETD . . . heated under nitrogen at 50.degree. C. for 18 h. The mixture was applied to a 10 cm column of silica **gel**, which was washed with 2:1 ethyl acetate-hexane. The eluate was concentrated and purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to afford 15.9 mg of the title compound as a white solid; Mass Spectrum (APCI). . .

DETD . . . and brine, saturated aqueous NaHCO.sub.3, dried over MgSO.sub.4, and concentrated. The residue was first filtered through a plug of silica **gel** and then purified by HPLC (Waters RCM, Prep Nova-Pak HR Silica, 25 mm.times.100 mm) using 8:4:1 hexane:t-butylmethylether:acetonitrile to afford 60.5. . .

DETD . . . heated under nitrogen at 50.degree. C. for 18 h. The mixture was applied to a 10 cm column of silica **gel**, which was washed

with 2:1 ethyl acetate-hexane. The eluate was concentrated and purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to afford 95 mg (88%) of the title compound as a white solid; .sup.1 H. . .

DETD . . . heated under nitrogen at 55.degree. C. for 14 h. The mixture was applied to a 10 cm column of silica **gel**, which was washed with 2:1 ethyl acetate-hexane. The eluate was concentrated and purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to afford 95 mg (88%) of the title compound as a white solid; .sup.1 H. . .

DETD . . . to 25.degree. C. for 14 hours. Volatiles were removed by vaccum and the residue was purified by chromatography on silica **gel** using 25% ethyl acetate-hexane to afford 100.2 mg of the title compound as a white solid; .sup.1 H NMR (CDCl.sub.3). . .

DETD . . . washed with 0.1M phosphate buffer (pH 7), then was dried over MgSO.sub.4 and concentrated. The residue was purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to afford 44.9 mg of the title compound as a white solid (46%); .sup.1 H. . .

DETD . . . temperature for 4 h, then was concentrated under reduced pressure. The residue was first filtered through a plug of silica **gel** and then purified by HPLC (Waters RCM, .mu. Porosil, 10 mm.times.10 cm) using a mixture of 9.6:6 (5:4:1 hexane-methyl tert-butyl. . .

DETD . . . was dissolved in a small amount of ethyl acetate/hexanes (1:1) (ca. 1 mL) and filtered through 30 g of silica **gel** eluting with 500 ml of ethyl acetate/hexanes (1:1). The first fractions, containing the Wilkinson-catalyst (approx. 50 mL) were discarried. The. . .

DETD . . . is dissolved in a small amount of ethyl acetate/hexanes (2:1) (ca. 1 mL) and filtered through 30 g of silica **gel** eluting with 500 ml of ethyl acetate/hexanes (2:1). The first fractions, containing the Wilkinson-catalyst (approx. 50 mL) is discarded. The. . .

DETD . . . heated under nitrogen at 50.degree. C. for 18 h. The mixture is applied to a 10 cm column of silica **gel**, which is washed with 2:1 ethyl acetate-hexane. The eluate is concentrated and purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to produce the title compound.

DETD . . . layer is washed with and brine, saturated aqueous NaHCO.sub.3, dried over MgSO.sub.4, and concentrated. The residue is purified by silica **gel** chromatography using S (hexane:t-butylmethylether:acetonitrile 8:4:1) to produce the title compound.

DETD . . . heated under nitrogen at 50.degree. C. for 18 h. The mixture is applied to a 10 cm column of silica **gel**, which was washed with 2:1 ethyl acetate-hexane. The eluate is concentrated and purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to produce the title compound.

DETD . . . heated under nitrogen at 55.degree. C. for 14 h. The mixture is applied to a 10 cm column of silica **gel**, which is washed with 2:1 ethyl acetate-hexane. The eluate is concentrated and purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to produce the title compound.

DETD . . . to 25.degree. C. for 14 hours. Volitiles are removed by vaccum and the residue is purified by chromatography on silica **gel** using 25% ethyl acetate-hexane to produce the title compound.

DETD . . . washed with 0.1M phosphate buffer (pH 7), then is dried over

MgSO.sub.4 and concentrated. The residue was purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to produce the title compound.

DETD . . . temperature for 4 h, then is concentrated under reduced pressure. The residue is first filtered through a plug of silica **gel** and then purified by HPLC (Waters RCM, .mu. Porosil, 10 mm.times.10 cm) using a mixture of 9.6:6 (5:4:1 hexane-methyl tert-butyl. . .

DETD . . . is dissolved in a small amount of ethyl acetate/hexanes (1:1) (ca. 1 mL) and filtered through 30 g of silica **gel** eluting with 500 ml of ethyl acetate/hexanes (1:1). The first fractions, containing the Wilkinson-catalyst (approx. 50 mL) are discarried. The. . .

CLM What is claimed is:

. . . pathogenic microorganisms, inflammatory and hyperproliferative skin diseases, psoriasis, atopical dermatitis, contact dermatitis, eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne, Alopecia areata, keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's. . .

14. The pharmaceutical formulation of claim 13, comprising in addition, a second immunosuppressive agent comprises **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester, cyclosporin, FK-506 and rapamycin.

. . . pathogenic microorganisms, inflammatory and hyperproliferative skin diseases, psoriasis, atopical dermatitis, contact dermatitis, eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne, Alopecia areata, keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's. . .

L9 ANSWER 21 OF 68 USPATFULL

AB The present invention comprises the method of selectively suppressing an immune response of a mammal to a particular alloantigen. The method includes several steps. One step is administering to a mammal an effective amount of UVB-radiation. Epidermal cell cultures, when subjected to UVA or UVB irradiation produce specific immunosuppressive factors. This UV-radiation is preferably UVA radiation (320 nm to 400 nm), or UVB-radiation (280 nm to 320 nm). It is demonstrated herein that UVA radiation results in in vitro cells producing a factor which selectively suppresses the CHS response in mammals, while UVB radiation selectively suppresses the DTH response in mammals. Another step of the inventive method involves desensitizing a mammal to a particular alloantigen. It has been determined that a mammal will become tolerant to a particular alloantigen once the subject mammal has been irradiated with a pre-determined wavelength of UVR and thereafter sensitized with the particular alloantigen. This may analogously be accomplished using factors from in vitro epidermal cell cultures.

AN 97:115242 USPATFULL

TI UVB-induced factor for immunosupression

IN Ullrich, Stephen E., Houston, TX, United States

PA Board of Regents, The University Of Texas System, Austin, TX, United States (U.S. corporation)

PI US 5696081 19971209 <--
AI US 1995-427629 19950424 (8)
RLI Continuation of Ser. No. US 1993-127272, filed on 24 Sep 1993, now abandoned which is a division of Ser. No. US 1991-768232, filed on 10 Oct 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-323615, filed on 14 Mar 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Krsek-Staples, Julie
LREP Arnold White & Durkee
CLMN Number of Claims: 2
ECL Exemplary Claim: 2
DRWN 13 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 2145
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5696081 19971209 <--
SUMM . . . wavelength of UVR to induce selective immunosuppression may have a marked advantage over the use of immunosuppressive drugs such as **azathioprine** or corticosteriods. Accordingly, the method of administering a sufficient amount of a pre-determined wavelength of UVR to selectively suppress an. . .
SUMM . . . .alpha.-D-mannopyranoside, indicating that the suppressive material is a glycoprotein. Analysis of the suppressive material and the control supernatants by polyacrylamide **gel** electrophoresis demonstrated a prominent band in the suppressive fractions that was not present in the non-suppressive fractions. The approximate molecular. . .
DRWD FIG. 5 shows the effect of UVB radiation and antigenic sensitization on **GVHD**. Lethally X-irradiated (850 rads) BALB/c mice were reconstituted with 5.times.10.sup.6 T cell-depleted C3H bone marrow cells (ATMB), anti-Thy 1.2 monoclonal. . .
DRWD FIG. 10 shows sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis (SDS-PAGE) analysis of the suppressive material eluted from conconavalin-A (Con A)-agarose columns. Equivalent amounts (200 ng) of the material eluted from the Con A-agarose columns were analyzed on 12.5% SDS-PAGE **gels** under reducing conditions. Lane 1 contained the UV mannoside eluate, lane 2 the UV glucoside eluate, lane 3 the control. . .
DETD Induction of Graft versus Host Disease (**GVHD**).
DETD **GVHD** was induced by using the procedure of Korngold and Sprent (26). Lethally X-irradiated (850 rads) BALB/c mice were reconstituted with. . .
DETD THE EFFECT OF UVB AND ALLOANTIGENIC SENSITIZATION ON **GVHD**
DETD The ability of UVB and alloantigenic sensitization to effect the survival of mice with lethal **GVHD** was examined. **GVHD** was induced by injecting lethally X-irradiated BALB/c mice with a mixture of T cell-depleted C3H bone marrow cells and mature. . . MST greater than 90 days was observed. Injection of normal spleen cells with the ATBM resulted in the induction of **GVHD** with an MST of 12 days. The use of spleen cells from mice exposed only to UVB (UVB spleen cells). . .
DETD A major problem in bone marrow transplantation is the induction of **GVHD**. Methods of reducing **GVHD** generally include histocompatibility matching between the donor and recipient, the use of immunosuppressive drugs, and the removal of T cells. . . the absence of any immunosuppressive drugs. The methods of the present invention

yield another method of reducing the incidence of **GVHD**.

DETD . . . the UV-irradiated or control keratinocytes (100 .mu.g total protein) were added to Con A bound to agarose (0.5 ml packed **gel** , Sigma Chemical Co.). The supernatants and the Con A-agarose were mixed together at 4.degree. for 30 minutes, and then added. . .

DETD . . . from the control nonirradiated (NR) keratinocytes and the UVirradiated keratinocytes (UV) were mixed wit Con A agarose (0.5 ml packed **gel**) and incubated at 4.degree. for 30 minutes. The **gel** was added to 1.0 ml syringes, and 5 ml of PBS was added to elute the unbound material. the bound. . .

L9 ANSWER 22 OF 68 USPATFULL

AB Substituted compounds of the FK-506 Type. These compounds are useful for the same or essentially the same purposes as FK-506 and are applied in the same or a similar manner. These compounds are immunosuppressants and useful for the treatment of autoimmune diseases, infectious diseases and/or the prevention of rejection of foreign organ transplants. Still other uses are described in the disclosure.

AN 97:112478 USPATFULL

TI O-aryl, O-alkyl, O-alkenyl and O-alkynyl-macrolides having immunosuppressive activity

IN Goulet, Mark, Westfield, NJ, United States
Organ, Helen M., Fanwood, NJ, United States
Parsons, William H., Edison, NJ, United States
Sinclair, Peter J., Highland Park, NJ, United States
Wong, Frederick, Glen Ridge, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5693648 19971202 <--
WO 9509857 19950413 <--

AI US 1996-619638 19960327 (8)
WO 1994-US11114 19940930
19960327 PCT 371 date
19960327 PCT 102(e) date

DT Utility

FS Granted

EXNAM Primary Examiner: Bond, Robert T.

LREP Yang, Mollie M., Rose, David L.

CLMN Number of Claims: 9

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 8531

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5693648 19971202 <--
WO 9509857 19950413 <--

SUMM . . . of foreign organ transplants, (e.g. bone marrow, kidney, liver, heart, skin, small-bowel, and pancreatic islet-cell transplants, including xeno transplants), the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as: psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus or Alopecia areata), male pattern

alopecia, alopecia senilis, reversible obstructive. . .

SUMM . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyper-proliferative skin diseases and of cutaneous manifestations of immunologically-mediated illness. A Fisons World patent application (PCT. . .

SUMM . . . onset diabetes, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .

SUMM . . . the suppression of in vitro immune systems (J. Antibiotics, 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).

SUMM . . . 3,644,364 and 4,098,791. Upjohn United States Patents (U.S. Pat. Nos. 4,139,619 and 4,596,812) discloses the use of minoxidil in the **topical** treatment of human baldness. Similarly, an Upjohn United States Patent (U.S. Pat. No. 5,026,691) discloses the use of minoxidil and an antiinflammatory agent for the treatment of patterned male and female alopecia. Japanese patent Kokai 61-260010 states that **topical** minoxidil formulations containing other specified agents may be prepared. An Upjohn WIPO patent application (PCT Publication No. WO 92/09259) discloses. . . University of Miami WIPO patent application (PCT Publication No. WO 92/12703) discloser a method of stimulating hair growth comprising the **topical** application of a phospholipid.

SUMM . . . chloroform, benzene, toluene and the like. The triarylbismuth(V) reagent can be used without purification or can be purified by silica **gel** chromatography. Triarylbismuthines may be prepared by the reaction of an appropriate aryl Grignard reagent with bismuth trichloride in an inert. . .

SUMM . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne Alopecia areata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .

SUMM . . . or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .

SUMM . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of **azathioprine** (AZA), brequinar sodium, deoxyspergualin (DSG), mizaribine, mycophenolic acid morpholino ester (RS-61443), cyclosporin and rapamycin.

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 3:4 EtOAc/hexanes to afford 46 mg of 17-ethyl-1,14-dihydroxy 12-[2'-(4"-phenyloxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-

azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1
H NMR, .sup.13 C NMR and mass. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The products were separated and purified by flash column chromatography on silica **gel** [eluted with 4:1 hexanes/acetone followed by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone] to yield 94 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-phenyloxy-3"-hydroxycyclohexyl)-1'-methyl-vinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 110 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-phenyloxy-4"-hydroxycyclo-hexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19, 21, 27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 3:1 hexanes/EtOAc) to afford 39 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-fluoro-phenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR and mass spectral. . .

DETD . . . Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was separated and purified two times by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to give 40 mg 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-chlorophenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/EtOAc) to give 47 mg 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methylphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 31 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-methylphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methylphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR. . .

DETD . . . dried over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (2:1 hexanes/acetone) to give 66 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-phenoxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis were. . .

DETD . . . over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified 3.times. by preparative TLC on silica **gel** (3:2 hexanes/acetone) to afford 35 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-phenoxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42 mg of 17-ethyl-1,14-dihydroxy-

12-[2'-(3"-(4'"-phenoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methyl-vinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C. . .

DETD . . . Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified 2 times by preparative TLC on silica **gel** (3:1 hexanes/acetone) to give 38 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-1-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR analysis was consistent with the desired structure).

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 3:1 hexanes/acetone) to yield 49 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(naphth-1-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 39 mg of 17-ethyl-1,14-dihydroxy-

12-[2'-(4"-(naphth-1-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-1,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (3:1 hexanes/acetone) to afford 32 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis were. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 3:1 hexanes/acetone) to give 63 mg of

17-ethyl-1,14-dihydroxy-12-[2'-(3"-(napth-2-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 49 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by two preparative thin layer chromatographys on silica **gel** (first chromatography eluted with 2:1 hexanes/acetone, isolated band at R.sub.f =0.26 second

chromatography eluted with 3.5% methanol/CH.sub.2 Cl.sub.2, isolated band. . .

DETD . . . The mixture was filtered and concentrated in vacuo. The triarylbismuthine is isolated and purified by flash column chromatography on silica **gel**.

DETD . . . dissolved in several milliliters of 4:1 hexanes/acetone plus small amount of CH.sub.2 Cl.sub.2. The solution was passed through a silica **gel** plug and eluted with 4:1 hexanes/acetone. The filtrate was concentrated in vacuo. The residue was dissolved in 4:1 hexanes/acetone plus small amount of CH.sub.2 Cl.sub.2 and passed through a second silica **gel** plug and eluted with 4:1 hexanes/acetone. The filtrate was concentrated in vacuo leaving 52 mg yellow residue that was used. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative thin layer chromatography on silica **gel** (eluted with 2:1 hexanes/acetone) to give 7.1 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-methoxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,-10,16-tetraone (R.sub.f =0.35) and 9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(6'"-methoxynaphth-2-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f. . .

DETD . . . (4 mL) was added bis(trifluoroacetoxy)iodobenzene (162 mg, 0.377 mmol). The mixture was stirred 5 minutes, then passed through a silica **gel** plug and eluted with EtOAc. The eluant was concentrated in vacuo. The residue was dissolved in CH.sub.2 Cl.sub.2 (4 mL). . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (2:1 hexanes/acetone) to afford 26.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methoxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.35). (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . (3 mL) was added bis(trifluoroacetoxy)iodobenzene (162 mg, 0.377 mmol). The mixture was stirred 5 minutes, then passed through a silica **gel** plug and eluted with EtOAc. The eluant was concentrated in vacuo. The residue was dissolved in CH.sub.2 Cl.sub.2 (4 mL). . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by radial chromatography on silica **gel** (2 mm plate eluted with 3:1 hexanes/acetone) and then by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 78.4 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'"-methoxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.40). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 47 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-tert-butyldimethylsilyloxynaphth-2-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f

=0.56).

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 44.2 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-hydroxynaphth-2-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.23). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by prepamtive TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 81 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-tert-buytldimethylsilyloxyphenyloxy)-3"-methoxycyclo-hexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ne-2,3,10,16-tetraone (R.sub.f =0.49). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 52 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-hydroxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.25). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 15.5 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methylthiophenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.47). (.sup.1 H NMR and mass spectral were. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 23.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4'-(2'"-methylphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.46). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by radial chromatography on silica **gel** (eluted with 3:1 hexanes/ethyl acetate) to afford 70.9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'"-methylphenyloxy)-3"-methoxycyclohexyl)-1'-methyl-vinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by radial chromatography on silica **gel** (eluted with 3.5% methanol/CH.sub.2 Cl.sub.2) and then purified by preparative TLC on silica **gel** (eluted with 3:1 hexanes/acetone) to afford 24.3 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'",4'"-dimethylphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral

analysis were consistent. . .
DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and
concentrated in vacuo. The products were separated by preparative TLC on
silica **gel** (2:1 hexanes/acetone). Each compound was repurified 2.times. by preparative TLC on silica **gel** (3:1 hexanes/acetone then 3.5% MeOH/CH.sub.2 Cl.sub.2) affording 23.4 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methoxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 28.4 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-methoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H. . .
DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and
concentrated in vacuo. The products were separated by preparative TLC on
silica **gel** (2:1 hexanes/acetone). Each compound was repurified 2.times. by preparative TLC on silica **gel** (2:1 hexanes/acetone then 3.5% MeOH/CH.sub.2 Cl.sub.2) affording 27 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'"-methoxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 35 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(3'"-methoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H. . .
DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and
concentrated in vacuo. The products were separated by preparative TLC on
silica **gel** (2:1 hexanes/acetone) affording 41.9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-tert-butyldimethylsilyloxyphenyloxy)-3"-hydroxy-cyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42.5 mg. of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-tert-butyldimethylsilyloxyphenyloxy)-4"-hydroxycyclo-hexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral. . .
DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) affording 25.7 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-hydroxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were consistent with. . .
DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and

concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) affording 23.9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-hydroxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis are consistent with. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated by preparative TLC on silica **gel** (2:1 hexanes/acetone) affording 39.8 mg of

17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-tert-butyldimethylsilyloxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 41.6 mg. of 17-ethyl-1,14-

dihydroxy-12-[2'-(3"-(6'"-tert-butyldimethylsilyloxynaphth-2-yl-oxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted 2.times. with 2:1 hexanes/acetone) affording 17 mg of
17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-hydroxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted 2.times. with 2:1 hexanes/acetone) affording 20.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(6'"-hydroxynaphth-2-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (3:2 EtOAc/hexanes) and a second preparative TLC (eluted 2.times. with 3:1 hexanes/acetone) affording 24.7 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(ethoxycarbomethoxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H. . .

DETD . . . dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 2:1 hexane/acetone to give 12 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(phenanthr-9-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR was consistent with the desired structure).

DETD . . . with anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in

vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 2:1 Hexane/Acetone) to give 37 mg of

17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'",4'"-methylenedioxyphenyloxy)-3"-methoxy-cyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . combined, dried with anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (eluted with 2:1 Hexane/Acetone) to give 14 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(2'",3'"-dihydrobenzofuran-5-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricycio[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone characterized by (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 3:1 Hexane/Acetone) to give 234 mg of 17-allyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . were combined, dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (eluted with 4% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 18 mg of
17-ethyl-14-dihydroxy-12-[2'-(4"-(1'",4'"-benzodioxane-

6-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and mass. . .

DETD . . . combined organic washes were dried with magnesium sulphate and concentrated. The crude residue was purified by column chromatography on silica **gel** eluting with 70% hexane:30% ethyl acetate to give the title Compounds A (93 mg) and B (102 mg) each as. . .

DETD . . . Cl.sub.2. The extracts were combined, dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. Purified by preparative TLC on silica **gel** (eluted with 7% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 22 mg of
17-ethyl-1,2,14-trihydroxy-12-[2'-(4"-(naphth-

2-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-3,10,16-trione (.sup.1 H NMR and mass. . .

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (156 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (17 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (10 mg).

DETD . . . at room temperature. After 1.5 hours, the mixture was filtered over Celite, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (19.5 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (21 mg 4"-ether; 17 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (15 mg 4"-ether; 16 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (11 mg 4"-ether; 13 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (14 mg 4"-ether; 12 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (24 mg 4"-ether; 21 mg 3"- ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (34 mg 4"-ether; 24 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (17 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (12 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (11 mg 4"-ether; 13 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (45 mg).

DETD . . . room temperature. After 30 minutes, the mixture was filtered over diatomacous earth, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (5.5 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1%

methanol) gave the title compound (13 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (9 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (8 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (16 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (10 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (17 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (20 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (33 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (34 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (19 mg).
DETD . . . at room temperature. After 45 minutes, the mixture was filtered over Celite, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (7.5 mg).
DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (6.8 mg). (.sup.1 H NMR was consistent with the desired structure).
DETD . . . at room temperature. After 25 minutes, the mixture was filtered over Celite, concentrated and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) to give the title compound (4.5 mg).
DETD . . . brine and the organic phase dried over magnesium sulfate. Removal of the solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (2.91 g). (.sup.1 H NMR was consistent with the desired structure).
DETD . . . sodium bicarbonate solution and the organic phase dried over

magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (1.51 g). (.sup.1 H NMR was consistent
with the desired structure).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ether:hexane (2:3)) gave the title compound (800 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride:hexane:methanol (10:2:1)) to give the title compound (300 mg) (.sup.1 H NMR was consistent. . .

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (151 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . extracted with ethyl acetate (3.times.5 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title
compound (3.5 mg).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride)
gave the title compound (2 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (320 mg). (.sup.1 H NMR was consistent
with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride:hexane:methanol (10:2:1)) to give the title compound (232 mg). (.sup.1 H NMR was consistent. . .

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (112 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . extracted with ethyl acetate (3.times.5 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title
compound (2.1 mg).

DETD . . . extracted with ethyl acetate (3.times.15 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1+1% methanol) to give the title compound (80.2 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . combined organics were washed with brine and dried over

magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (24 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (4 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (92 mg).

DETD . . . combined organics are washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** gives the title compound.

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (190 mg).

DETD . . . ethyl acetate. The combined organics were dried over magnesium sulfate, concentrated in vacuo and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1) to give the title compound (50 mg).

DETD . . . The organics were dried by passage through a magnesium sulfate plug and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol then (1:1+1% methanol) to give the title compound (13 mg).

DETD . . . mg) and the reaction stirred at room temperature. After 30 minutes the mixture was filtered through a small diatomaceous earth/silica **gel** plug and the filtrate concentrated in vacuo. Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (10 mg).

DETD . . . and brine. The combined organics were dried over magnesium sulfate and concentrated in vacuo. Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the desired product (145 mg).

DETD . . . organics were dried by passage through a magnesium sulfate plug, concentrated in vacuo and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1) 1% methanol) to give the title compound (43 mg).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (6 mg).

DETD . . . and the organic portion washed with brine, dried over magnesium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (3:2) to give the title compound (8.4 g)

DETD . . . sodium bicarbonate, brine, and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (10% acetone in hexane) gave the title compounds (3" ether: 1.81 g, 4" ether: 1.20 g).

DETD . . . and the organic portion washed with brine, dried over magnesium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1+1% methanol) to give the title compound (316 mg).

DETD . . . (5.5 mg), and the mixture stirred at room temperature. After 15

minutes, the mixture was filtered through a small silica **gel** column, washed with ethyl acetate, and the concentrated organics purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (282 mg).

DETD . . . washed with water. The organic portion was dried over sodium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (4:1) +1% methanol +0.5% acetic acid) to give the title compound (43 mg).

DETD . . . colored persisted. The mixture was then warmed to room temperature, concentrated in vacuo, and purified by flash chromatography on silica **gel** (acetone:hexane (1:2)) to give the title compound (5.5 mg).

DETD . . . at room temperature for 12 hours. At this time the mixture was concentrared and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (43 mg).

DETD . . . ml), and the combined organic portions washed with brine, dried over magenesium sulfate and purified by flash chromatography on silica **gel** (2% methanol in methylene chloride followed by 2% methanol in methylene chloride+0.5% acetic acid) to give the title compound (255. . .

DETD . . . sodium bicarbonate. The organic portion was dried over magnesium sulfate, concentrated in vacuo, and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then (2:1)+1% methanol) to give the title compound (14 mg).

DETD . . . extracted with ethyl acetate, and the organics dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (5 mg).

DETD . . . with ethyl acetate, and the organic portion dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) gave the title compound (74 mg).

DETD . . . with ethyl acetate, and the organic portion dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol, in methylene chloride) gave the title compound (10 mg).

DETD . . . (2 ml) dropwise. The reaction mixture was stirred for 15 minutes after the addition and then filtered through a silica **gel** pad washing with ethyl acetate. The filtrate was concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired product (188 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 50% hexane:50% ethyl acetate to give the title compound (102 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired product (216 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 70% hexane:30% ethyl acetate to give the title compound (11 mg).

DETD . . . acetate. The organic extracts were dried (MgSO.sub.4) and

concentrated and the crude material was purified by column chromatography on silica **gel** eluting with 65% hexane:35% ethyl acetate to give the desired product (22 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 50% hexane:50% ethyl acetate to give the title compound (15 mg).

DETD . . . stirred at room temperature for 48 hours. The reaction was then diluted with ethyl acetate and filtered through a silica **gel** pad. The filtrate was concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired compound (12.6 mg).

DETD . . . brine and extracted with ethyl acetate. The organic extracts were dried (MgSO.sub.4), concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired compound (27 mg).

DETD . . . washed with saturated sodium chloride solution, and the organic portion dried over magnesium sulfate. Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) followed by silica **gel** preparative TLC (acetone:hexane 2:8) gave the title compound (2.8 mg).

DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2 minutes at 4.degree. C. Cold medium was added and cells were again. . .

L9 ANSWER 23 OF 68 USPATFULL

AB A method for treating inflammatory bowel disease in a mammal that includes administering to the mammal and effective amount of spiperone or a spiperone derivative or a pharamaceutically acceptable salt thereof.

AN 97:112475 USPATFULL

TI Use of spiperone or spiperone derivatives as immunosuppressant agents

IN Sharpe, Richard J., Gloucester, MA, United States
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Galli, Stephen J., Winchester, MA, United States
Meltzer, Peter C., Lexington, MA, United States
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Sard, Howard P., Arlington, MA, United States

PA Beth Israel Deaconess Medical Center, Inc., Boston, MA, United States (U.S. corporation)

PI US 5693645 19971202 <--
WO 9312789 19930708 <--

AI US 1994-256158 19940831 (8)
WO 1992-US11205 19921223
19940831 PCT 371 date
19940831 PCT 102(e) date

DT Utility

FS Granted

EXNAM Primary Examiner: Jordan, Kimberly

LREP Kilpatrick Stockton LLP

CLMN Number of Claims: 21

ECL Exemplary Claim: 1

DRWN 16 Drawing Figure(s); 8 Drawing Page(s)

LN.CNT 1340

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5693645 19971202 <--
WO 9312789 19930708 <--

SUMM Cutaneous contact hypersensitivity and asthma are just two examples of **topical** immune responses that can be associated with significant morbidity. Others include atopic dermatitis, eczema, psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia reactions, allergic responses due to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, lichen planus, asthma, allergic asthma, cutaneous lupus erythematosus, dry eye associated with Sjogren's Syndrome,. . .

SUMM . . . agents with partial utility for treating some of the above conditions include psoralen plus ultraviolet A (PUVA), cyclosporin A, or **azathioprine**, but the risk-to-benefit ratios for these agents is unfavorable for most of the conditions described above.

SUMM Various therapeutics that have been utilized as systemic immunosuppressants include steroid hormones, anti-metabolites such as methotrexate and **azathioprine**, cyclosporine, alkylating agents such as cyclophosphamide and busulfan, and certain antibiotics. However, there still remains a strong need to provide. . .

SUMM There remains a need for compounds and methods for the treatment of patients in need of **topical** or systemic immmunosupression.

SUMM It is therefore an object of the present invention to provide a method and compositions for the **topical** or systemic suppression pathogenic immune responses.

SUMM A method for the **topical** or systemic immunosuppression of a human or other mammal in need of immunosuppression is disclosed wherein the mammal is treated. . . spiperone or a spiperone derivative, or its pharmaceutically acceptable salt, optionally in a pharmaceutically-acceptable diluent or carrier for systemic or **topical** application.

SUMM Spiperone and its active derivatives are useful as **topical** agents in treating contact dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease (inflammatory bowel disease), **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions. The novel. . .

DRWD . . . contact hypersensitivity reactions. These data (mean.+-.SEM) are from the same mice whose ea thickness measurements are presented in FIG. 5. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**p<0.01).

DRWD FIGS. 8a,b,c--Effect of **topical** treatment with spiperone on leukocyte infiltration associated with oxazolone-induced contact hypersensitivity reactions. These data (mean.+-.SEM) are from the same mice. . . are presented in FIGS. 7a,b,c. Biopsies were performed 24 hours (a, b) or 46 hours (c) after application of oxazalone. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**=p<0.01). In FIG. 8a, the slight. . .

DRWD FIG. 10--Effect of **topical** treatment with spiperone on leukocyte infiltration associated with DNFB-induced contact hypersensitivity reactions. These data (mean.+-.SEM) are from the same

mice whose ear thickness measurements are presented in FIG. 9. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**p<0.01). The slight effect of treatment. . .

DETD Mammals, and specifically humans, suffering from pathogenic immune responses can be treated by **topical** or systemic administration to the patient of an effective amount of spiperone or its derivative or pharmaceutically acceptable salt, optionally. . .

DETD . . . to 500 mg/kg of body weight per day as a single daily dose or divided daily doses. Typical dosages for **topical** application are those ranging from 0.001 to 100% by weight of the active compound. In general, local immunosuppression can be. . .

DETD Solutions or suspensions used for parenteral, intradermal, subcutaneous, Cr **topical** application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,. . .

DETD Suitable vehicles or carriers for **topical** application can be prepared by conventional techniques, such as lotions, suspensions, **ointments**, **creams**, **gels**, tinctures, sprays, powders, pastes, slow-release transdermal patches, suppositories for application to rectal, vaginal, nasal or oral mucosa. In addition to the other materials listed above for systemic administration, thickening agents, emollients, and stabilizers can be used to prepare **topical** compositions. Examples of thickening agents include petrolatum, beeswax, xanthan gum, or polyethylene, humectants such as sorbitol, emollients such as mineral oil, lanolin and its derivatives, or squalene. A number of solutions and **ointments** are commercially available, especially for ophthalmic applications.

DETD . . . the tissue swelling and the leukocyte infiltration associated with the elicitation phase of contact hypersensitivity to either oxazolone or dinitrofluorobenzene. **Topical** treatment with spiperone also suppressed the sensitization phase of contact sensitivity. However, mice treated topically with spiperone, unlike those treated. . .

DETD **Topical** Spiperone Treatment--To test whether spiperone affected the sensitization phase of contact hypersensitivity, 50 .mu.l of 0.08% spiperone in propylene glycol. . .

DETD . . . infiltration at sites of hapten challenge than did vehicle-treated mice (p<0.01 for either comparison). These data show that treatment with **topical** spiperone can effectively inhibit the sensitization phase of cutaneous contact hypersensitivity.

DETD Effects of **Topical** Spiperone on Expression of Contact Hypersensitivity--For these experiments, both ears of each mouse were challenged for elicitation of contact hypersensitivity. . . in vehicle, applied epicutaneously to both surfaces. The right ears of control mice were similarly treated, but with vehicle alone. **Topical** administration of a 4.0% suspension of spiperone in absolute ethanol, propylene glycol, and olive oil one hour after hapten challenge. . .

DETD Although **topical** application of spiperone was extremely effective in diminishing both the tissue swelling and the leukocyte infiltration associated with contact hypersensitivity. . .

DETD To evaluate the effect of **topical** treatment with spiperone on contact hypersensitivity reactions elicited with a different hapten, the effect of **topical** treatment with a 0.5% suspension of spiperone on the contact hypersensitivity reactions elicited with DNFB

was examined. **Topical** treatment with spiperone significantly diminished the tissue swelling associated with reactions to DNFB (by 45%, FIG. 9) and had an. . .

DETD Mice were sensitized to oxazolone as described in example 1. Three days later, slow release indomethacin **pellets** (0.05 mg, 3 week release) were implanted subcutaneously under light ether anesthesia. The dose of indomethacin delivered by these **pellets** has been previously shown to completely block prostaglandin synthesis in mice, by Jun, D. D., et al., J. Invest. Dermatol.. . .

DETD . . . volumes of 50 mM Tris HCl buffer pH 7.7 at 25.degree. C. and centrifuged at 49,000.times.g for 10 min. The **pellet** is resuspended in fresh buffer and incubated at 37.degree. C. for 10 min. After the final centrifugation, the **pellet** is resuspended in 80 volumes of Krebs-HEPES buffer (25 mM HEPES, 118 mM NaCl, 5 mM KCl, 2.5 mM CaCl.sub.2,. . .

L9 ANSWER 24 OF 68 USPATFULL

AB The present invention provides methods of treating a subject suffering from adverse effects, complications or conditions, associated with or resulting from corneal transplantation, by **topical** administration of suitable ophthalmic preparations of bactericidal/permeability-increasing (BPI) protein products.

AN 97:104444 USPATFULL

TI Methods of treating conditions associated with corneal transplantation

IN Scannon, Patrick J., San Francisco, CA, United States

PA Xoma Corporation, Berkeley, CA, United States (U.S. corporation)

PI US 5686414 19971111 <--

AI US 1995-557287 19951114 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Carlson, Karen C.

LREP McAndrews, Held & Malloy, Ltd.

CLMN Number of Claims: 9

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 931

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5686414 19971111 <--

AB . . . methods of treating a subject suffering from adverse effects, complications or conditions, associated with or resulting from corneal transplantation, by **topical** administration of suitable ophthalmic preparations of bactericidal/permeability-increasing (BPI) protein products.

SUMM . . . methods of treating a subject suffering from adverse effects, complications or conditions, associated with or resulting from corneal transplantation, by **topical** administration of bactericidal/permeability-increasing (BPI) protein products.

SUMM . . . uncontrolled glaucoma, anterior synechiae, uveitis, and recurrent or progressive forms of conjunctival inflammation, such as acne rosacea and ocular cicatricial **pemphigoid**. In general, the most favorable prognosis attaches to transplants effected in response to localized corneal scars, keratoconus and cornea/dystrophies.

SUMM . . . or treating rejection and its associated conditions or complications have been limited principally to administration of immunosuppressant corticosteroids. In fact, **topical** corticosteroids have been the mainstay of therapy in the prevention and treatment of corneal allograft rejection in humans and treatment. . .

the specifics of postoperative corticosteroid therapy. Steroids are sometimes continued anti the time of suture removal, while some clinicians continue **topical** therapy in small doses for 1 or more years (sometimes indefinitely). Patients must be alerted to the earliest symptoms of graft rejection. For "mild" signs of rejection it may be sufficient to administer **topical** steroids every 3 hours with careful follow-up every second day to monitor the effect. In such cases, treatment would likely. . . local corticosteroid treatment may be given early in the course of a reaction. This usually consists of hourly applications of **topical** steroids and even periocular injections of depot preparations.

SUMM . . . set of complications and risks. In particular, ocular toxicity and localized side effects of corticosteroids present significant problems. For example, **topical** corticosteroids can cause ocular hypertension or cataracts, enhance secondary bacterial, fungal or vital infections of the ocular surface due to. . . the clinical course of these infections. In one study, 68-100% of patients developing microbial keratitis in grafts were reportedly using **topical** corticosteroid drops at the time the infection occurred. It is known that corticosteroid drops specifically impair the local host-defense mechanisms. . .

SUMM In cases of infection associated with steroid therapy following transplantation, chronic **topical** antibiotic administration may allow resistant organisms to emerge and affect the development and course of microbial keratitis following transplant. In. . .

SUMM . . . of antibiotics are continually being investigated, but thus far have met with little success. Drugs such as antilymphocyte serum and **azathioprine** have been used experimentally, but in general have been considered too dangerous for routine use in clinical situations. While immunologic. . .

SUMM The present invention provides novel methods for treating corneal transplant patients through **topical** administration to the cornea of the patient of a bactericidal/permeability-increasing (BPI) product in an mount effective to reduce the incidence. . .

SUMM . . . This aspect of the invention contemplates concurrent administration of BPI protein product with any antimicrobial agent or combinations thereof for **topical** use in the eye including: antibacterial agents such as gentamicin, tobramycin, bacitracin, chloramphenicol, ciprofloxacin, ofloxaein, norfloxacin, erythromycin, bacitracin/neomyein/polymyxin B, sulfisoxazole,. . .

SUMM . . . BPI protein product is preferably administered topically, to the corneal surface. The BPI protein product may be additionally administered systemically. **Topical** routes include administration preferably in the form of ophthalmic drops, **ointments**, **gels** or salves. Other **topical** routes include irrigation fluids (for, e.g., irrigation of wounds). Those skilled in the art can readily optimize effective ophthalmic dosages. . .

DETD . . . administration on allograft rejection is evaluated in a corneal transplantation allogenic rabbit model when administered alone or concurrently with a **topical** corticosteroid and/or antimicrobial agent.

DETD . . . are anesthetized by intramuscular injection of 0.5-0.7 mL/kg rodent cocktail (100 mg/mL ketamine, 20 mg/mL xylazine, and 10 mg/mL acepromazine). **Topical** anaesthetic drops of proparacaine

hydrochloride (0.5% Ophthaine, Bristol-Myers Squibb) are instilled into the animals eye together with drops of cyclopentolate. . .

DETD . . . limbus in order to encourage vascularization, with no attempt to bury the knot. At the end of the procedure, chloramphenicol **ointment** is placed on the operated eye. Alternatively, or as an adjunct to chloramphenicol a BPI protein product ophthalmic solution is.

. .

DETD Postoperatively, all animals receive atropine drops (1%, Atropine sulfate, Bausch & Lomb, Tampa, Fla.) and chloramphenicol **ointment** (1.0%, Bausch & Lomb, Tampa, Fla.) daily until the removal of the corneal suture on day 14. On the first. . .

DETD . . . BPI protein product administration is evaluated in a corneal xenograft transplantation model when administered alone or in combination with a **topical** corticosteroid and/or antimicrobial agent.

DETD . . . are anesthetized by intramuscular injection of 0.5-0.7 mL/kg rodent cocktail (100 mg/mL ketamine, 20 mg/mL xylazine, and 10 mg/mL acepromazine). **Topical** anaesthetic drops of proparacaine hydrochloride (0.5% Ophthaine, Bristol-Myers Squibb) are instilled into the animals eye together with drops of cyclopentolate. . .

L9 ANSWER 25 OF 68 USPATFULL

AB The compounds of Formula I ##STR1## are useful as immunosuppressive agents.

AN 97:96896 USPATFULL

TI Triterpene derivatives with immunosuppressant activity

IN Baker, Robert K., Cranford, NJ, United States
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Bao, Jianming, Westfield, NJ, United States
Parsons, William H., Belle Mead, NJ, United States
Rupprecht, Kathleen M., Cranford, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5679705 19971021 <--

AI US 1996-734247 19961016 (8)

PRAI US 1995-6085P 19951031 (60)

DT Utility

FS Granted

EXNAM Primary Examiner: Dentz, Bernard

LREP Camara, Valerie J., Daniel, Mark R.

CLMN Number of Claims: 19

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 2850

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5679705 19971021 <--

SUMM . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, Graves ophthalmopathy and asthma.

DETD . . . pathogenic microorganisms, inflammatory and hyperproliferative skin diseases, psoriasis, atopical dermatitis, contact dermatitis, eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne, Alopecia areata, keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's. . .

DETD . . . as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises and further eczematous dermatitises,

seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise). . .

DETD . . . as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise). . .

DETD . . . or more immunosuppressant agents. These immunosuppressant agents within the scope of this invention include, but are not limited to, IMUREK.RTM. **azathioprine** sodium, brequinar sodium, SPANIDIN.RTM. gusperimus trihydrochloride (also known as deoxyspergualin), mizoribine (also known as bredinin), CELLCEPT.RTM. mycophenolate mofetil, NEORAL.RTM. Cyclosporin. . .

DETD . . . ingredient compound with the site of action in the body of a warm-blooded animal. For example, administration, can be oral, **topical**, including transdermal, ocular, buccal, intranasal, inhalation, intravaginal, rectal, intracisternal and parenteral. The term "parenteral" as used herein refers to modes. . .

DETD . . . as dispersions, suspensions or solutions. Other dosages forms that can also be used to administer the active ingredient as an **ointment**, **cream**, drops, transdermal patch or powder for **topical** administration, as an ophthalmic solution or suspension formation, i.e., eye drops, for ocular administration, as an aerosol spray or powder composition for inhalation or intranasal administration, or as a **cream**, **ointment**, spray or suppository for rectal or vaginal administration.

DETD . . . This was first fractionated by preparative thin layer chromatography (TLC) on a 20 cm by 20 cm E. Merck silica **gel** 60F.sub.254 plate of 1 mm thickness using methylene chloride-ethyl acetate 1:1 (v/v) as solvent, then by high performance liquid chromatography. . .

DETD Homogeneity of the preparations was ascertained in several TLC systems, such as E. Merck silica **gel** 60F.sub.254, methylene chloride-ethyl acetate 1:1, Rf 1(a) 0.4, Rf 1(b) 0.3; Whatman KC.sub.18, methanol-water 9:1, Rf 1(a) 0.65, Rf 1(b). . .

DETD Partial purification of the methylene chloride extract was achieved by column chromatography on E. Merck silica **gel** 60 (120 ml), eluting with a step gradient of ethyl acetate in methylene chloride. The step gradient was designed so. . . afforded 100 mg and 20 mg respectively of 1(a) and 1(b) after crystallization from methanol. Later-eluting fractions from the silica **gel** column above were found to contain at least two related compounds based on UV spectra and color reactions on TLC. . .

DETD . . . chloride each time. The pooled methylene chloride extracts are evaporated down and fractionation proceeds by repeated column chromatography on silica **gel**. One employs methylene chloride-methanol 97:3 in a first step; the mixed compounds of Formula 1(a) and 1(b) thus obtained are resolved by chromatographing on fresh silica **gel** eluted with methylene chloride-ethyl acetate 3: 1. Volume of elution for the compound of Formula 1(a) ranges from about 2. . .

DETD . . . dissolved in a small mount of ethyl acetate/hexanes (2:1) (ca.

1 mL) and filtered through 30 g of silica **gel** eluting with 500 ml of ethyl acetate/hexanes (2:1). The first fractions, containing the Wilkinson-catalyst (approx. 50 mL) were discarded. The. . .

DETD . . . layer was washed with and brine, saturated aqueous NaHCO.sub.3, dried over MgSO.sub.4, and concentrated. The residue was purified by silica **gel** chromatography using S (hexane:t-butylmethylether:acetonitrile 8:4:1) to afford 60.5 mg (29%) of the title compound as a white solid; .sup.1 H. . .

DETD . . . hydrochloric acid and saturated aqueous sodium chloride then was dried over MgSO.sub.4 and concentrated. The residue was purified by silica **gel** chromatography with 1:1 ethyl acetate-hexane to afford 41.8 mg of the title compound as a white solid (80%); .sup.1 H. . .

DETD . . . to 25.degree. C. for 14 hours. Volatiles were removed by vacuum and the residue was purified by chromatography on silica **gel** using 25% ethyl acetate-hexane to afford 13.8 mg (100%) of the title compound as a white solid; .sup.1 H NMR. . .

DETD . . . with CH.sub.2 Cl.sub.2 and was filtered through celite. Upon evaporation of solvent, the residue was purified by chromatography on silica **gel** using 50% ethyl acetate-hexane to afford 4.0 mg (20%) of the Z isomer and 5.4 mg of the E isomer. . .

DETD . . . washed with 0.1M phosphate buffer (pH 7), then was dried over MgSO.sub.4 and concentrated. The residue was purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to afford 44.9 mg of the title compound as a white solid (46%); .sup.1 H. . .

DETD . . . temperature for 4 h, then was concentrated under reduced pressure. The residue was first filtered through a plug of silica **gel** and then purified by HPLC (Waters RCM, .mu.Porosil, 10 mm.times.10 cm) using a mixture of 9.6:6 (5:4:1 hexane-methyl tert-butyl ether-acetonitrile:hexane). . .

DETD . . . was dissolved in a small amount of ethyl acetate/hexanes (1:1) (ca. 1 mL) and filtered through 30 g of silica **gel** eluting with 500ml of ethyl acetate/hexanes (1:1). The first fractions, containing the Wilkinson-catalyst (approx. 50 mL) were discarded. The fractions. . .

DETD . . . is dissolved in a small amount of ethyl acetate/hexanes (2:1) (ca. 1 mL) and filtered through 30 g of silica **gel** eluting with 500 ml of ethyl acetate/hexanes (2:1). The first fractions, containing the Wilkinson-catalyst (approx. 50 mL) are discarded. The. .

DETD . . . layer is washed with and brine, saturated aqueous NaHCO.sub.3, dried over MgSO.sub.4, and concentrated. The residue is purified by silica **gel** chromatography using S (hexane:t-butylmethylether:acetonitrile 8:4:1) to produce the title compound.

DETD . . . hydrochloric acid and saturated aqueous sodium chloride then is dried over MgSO.sub.4 and concentrated. The residue is purified by silica **gel** chromatography with 1:1 ethyl acetate-hexane to produce the title compound.

DETD . . . to 25.degree. C. for 14 hours. Volitiles are removed by vacuum and the residue is purified by chromatography on silica **gel** using 25% ethyl acetate-hexane to produce the title compound.

DETD . . . with CH.sub.2 Cl.sub.2 and is filtered through celite. Upon evaporation of solvent, the residue is purified by chromatography on silica **gel** using 50% ethyl acetate-hexane to separate the Z isomer and the E isomer.

DETD . . . washed with 0.1M phosphate buffer (pH 7), then is dried over MgSO.sub.4 and concentrated. The residue is purified by silica **gel** chromatography with 2:1 ethyl acetate-hexane to produce the title compound.

DETD . . . temperature for 4 h, then is concentrated under reduced pressure. The residue is first filtered through a plug of silica **gel** and then purified by HPLC (Waters RCM, .mu.Porosil, 10 mm.times.10 cm) using a mixture of 9.6:6 (5:4:1 hexane-methyl tert-butyl ether-acetonitrile:hexane). . .

DETD . . . dissolved in a small amount of ethyl acetate/hexanes (1:1) (ca. 1 mL) and is filtered through 30 g of silica **gel** eluting with 500ml of ethyl acetate/hexanes (1:1). The first fractions, containing the Wilkinson-catalyst (approx. 50 mL) are discarded. The fractions. . .

CLM What is claimed is:

. . . pathogenic microorganisms, inflammatory and hyperproliferative skin diseases, psoriasis, atopical dermatitis, contact dermatitis, eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne, Alopecia areata, keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's. . .

14. The pharmaceutical formulation of claim 13, comprising in addition, a second immunosuppressive agent comprising **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester, cyclosporin, FK-506 and rapamycin.

. . . pathogenic microorganisms, inflammatory and hyperproliferative skin diseases, psoriasis, atopical dermatitis, contact dermatitis, eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne, Alopecia areata, keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's. . .

L9 ANSWER 26 OF 68 USPATFULL

AB Novel macrolide compounds of the formula ##STR1## and pharmaceutically acceptable salts, esters, amides and prodrugs thereof, processes for the preparation of the compounds of the invention, intermediates useful in these processes, a pharmaceutical composition, and a method of treating immunomodulatory disorders are disclosed.

AN 97:88986 USPATFULL

TI Macrolide immunomodulators

IN Or, Yat Sun, Libertyville, IL, United States
Luly, Jay R., Libertyville, IL, United States
Wagner, Rolf, Gurnee, IL, United States

PA Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

PI US 5672605 19970930 <--

AI US 1995-424931 19950419 (8)

RLI Division of Ser. No. US 1994-327391, filed on 26 Oct 1994 which is a continuation-in-part of Ser. No. US 1993-155064, filed on 19 Nov 1993, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Bond, Robert T.

LREP Crowley, Steven R.
CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 5847
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5672605 19970930 <--
SUMM . . . is beneficial as well. These other immunosuppressant agents include but are not limited to FK-506, rapamycin, cyclosporin A, mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide.
SUMM . . . OH and R.sup.9 is hydrogen with fluorosulfonyl anhydride or trifluoromethylsulfonyl anhydride, followed by reaction of the resulting sulfonate with silica **gel** or an appropriate base to produce the enol ether, followed by hydrolysis of the enol ether; or
SUMM . . . of formula I where R.sup.8 is --OSO.sub.2 F or --OSO.sub.2 CF.sub.3 and R.sup.9 is hydrogen, in the presence of silica **gel** or appropriate mild acid under conditions suitable for the production of the desired product and hydrolysis of the enol ether.
SUMM A suitable reagent for the dehydration of an activated alcohol is silica **gel** or triethylamine. The reaction may be carried out in a solvent which does not adversely affect the reaction (e.g. diethyl. . .
SUMM In process (mm), a suitable acid for the rearrangement of the activated alcohol is silica **gel**. The reaction may be carried out in a solvent which does not adversely affect the reaction (e.g. diethyl ether, dichloromethane,. . .
DETD . . . N hydrochloric acid. The organic phase was washed once with saturated brine, dried over magnesium sulfate and filtered through silica **gel** (2 g) eluting with ether. The solvent was removed in vacuo, and the product was stored in the freezer.
DETD . . . was washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product was purified by silica **gel** chromatography (20 g) eluting with 20% acetoneexanes to afford 0.72 g of the title compound. MS (FAB) m/z: M+K=1117.
DETD . . . mixture was allowed to warm to room temperature and stirred for 2 hours. The reaction mixture was purified by silica **gel** chromatography (70 g) eluting with 25% acetone/hexanes to give 343.2 mg of the title compound. m.p. 115.degree.-199.degree. C. MS (FAB)m/z:. . .
DETD . . . is washed once with brine, dried over magnesium sulfate, and solvent removed in vauco. The product is purified by silica **gel** chromatography eluting with 30% acetone/hexanes.
DETD . . . 20 mL of water and 20 mL of saturated NaCl solution, dried over magnesium sulfate and passed through a silica **gel** plug eluting with cold ether. The solvent was removed in vacuo, and the residue was dissolved in 10 mL of. . . mL of saturated NaCl solution, dried over MgSO.sub.4 and concentrated in vacuo. The residue obtained was chromatogrpahed on a silica **gel** (15 g) column eluting with 4% isopropanol in dichlormethane to give 271 mg of the title compound. m.p. 90.degree.-93.degree. C.. . .
DETD . . . N hydrochloric acid. The organic phase was washed once with saturated brine, dried over magnesium sulfate and filtered through

silica **gel** (2 g) eluting with ether. The solvent was removed in vacuo, and the product was stored in the freezer.

DETD . . . The organic phase was washed with saturated NaCl solution, dried over MgSO.sub.4 and passed through a short column of silica **gel** (10 g). The partially purified compound was further purified by HPLC (Rainin Microsorb silica **gel**) eluting with 75% acetone in hexane to afford the title compound. m.p. 105.degree.-109.degree. C. MS (FAB) m: M+K=1039. Selected CMR. . .

DETD . . . is added and stirred for another 0.5 hour. The solids are filtered off and the product is purified by silica **gel** chromatography.

DETD Silica **gel** (25 g) was added to a solution of the compound resulting from Example 13 (prepared from 0.53 g of rapamycin). . . then removed in vacuo, and the resulting powder was refrigerated for 8 days at 8.degree. C. The product on silica **gel** was eluted with acetone and the solvent removed in vacuo. The crude product was purified by HPLC (Rainin Microsorb silica **gel**) eluting with 30% acetone/hexanes. MS (FAB) m/z: M+K=920.

DETD Silica **gel** (25 g) was added to a solution of the compound resulting from Example 13 (prepared from 0.53 g of rapamycin). . . then removed in vacuo, and the resulting powder was refrigerated for 8 days at 8.degree. C. The product on silica **gel** was eluted with acetone and solvent removed in vacuo. The crude product was purified by HPLC (Rainin Microsorb silica **gel**) eluting with 30% acetone/hexanes. MS (FAB) m/z: M+K=934.

DETD The title compound was isolated from the reaction mixture on silica **gel** of Example 36. MS (FAB) m/z: M+K=934.

DETD . . . once with saturated sodium chloride solution, dried over MgSO.sub.4 and concentrated in vacuo. The residue was purified on a silica **gel** column eluting with 1:1 acetone-hexane to give 380 mg of partially purified material which was further purified by HPLC eluting. . .

DETD . . . a nitrogen atmosphere and then partitioned between ether and 0.1 N HCl. The organic phase was passed through a silica **gel** plug eluting with Et.sub.2 O. This activated intermediate was dissolved in methylene chloride (8 mL), cooled to -78.degree. C., and. . . O and 0.1 N HCl. The organic phase was concentrated in vacuo, and the residue obtained purified on a silica **gel** column eluting with 4% isopropanol in methylene chloride to give 159 mg of the title compound. m.p. 111.degree.-116.degree. C. MS. . .

DETD . . . continuing 30 minutes after complete addition. The solvent is removed in vacuo and the residue purified by HPLC on silica **gel** . Fractions containing desired product are pooled, and concentrated, to constant weight under high vacuum to give the desired product.

DETD . . . continuing 30 minutes after complete addition. The solvent is removed in vacuo and the residue purified by HPLC on silica **gel** . Fractions containing desired product are pooled, and concentrated, to constant weight under high vacuum to give the desired product.

DETD . . . C. for an additional 24 hours. The solvent is removed in vacuo and the residue purified by chromatography on silica **gel** to provide the title compound.

DETD . . . of piperidine. After complete consumption of starting material, as evidenced by TLC, the material is purified by chromatography on silica **gel** to provide the title compound.

DETD . . . (2 g) was added and stirring was continued for 30 minutes. The crude mixture was then passed through a silica **gel** column. This partially purified material was rechromatographed on silica **gel** eluting with 35% acetone in hexane to obtain the title

compound (380 mg, 40%) which was recrystallized from ether. m.p.. . .
DETD . . . between Et.sub.2 O and water. The organic phase was dried over magnesium sulfate, concentrated in vacuo and purified by silica **gel** chromatography to afford the title compound. MS (FAB) m/z: M+K=951.
DETD . . . once with brine, dried over magnesium sulfate and the solvent removed in vacuo. The crude product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.
DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.
DETD . . . washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The crude product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.
DETD . . . mL) at 0.degree. C. and refregirated overnight. Pyridine is removed in vacuo, and the crude mixture is purified by silica **gel** chromatography eluting with 65% acetone in hexanes.
DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.
DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.
DETD . . . washed once with brine, dried over magnesium sulfate and the solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.
DETD . . . washed once with brine, dried over magnesium sulfate and the solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.
DETD . . . and water. The organic phase was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by silica **gel** chromatography to give the title compound (189 mg). m.p. 105.degree.-111.degree. C. MS (FAB) m: M +K=968.
DETD . . . stirring at room temperature for 16 hours, the solvent is removed in vacuo, and the product is purified by silica **gel** chromatogrphy eluting with 5% isopropanol in dichloromethane.
DETD . . . stirring at room temperature for 5 hours, the solvent is removed in vacuo, and the product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.
DETD . . . 0.5 mL of methanol. The reaction mixture was stirred at room temperature for 36 hours and then chromatographed on silica **gel** eluting with 50% acetone in hexanes to afford 0.277 g of the title compound. m.p. 126.degree.-131.degree. C. MS (FAB) m/z:. . .
DETD . . . g) in dichloromethane-tetrahydrofuran (1:1, 4 mL). The reaction mixture was stirred at room temperature overnight and then chromatographed on silica **gel** eluting with 50% acetone in hexanes to afford 0.45 g of the title compound. m.p. 101.degree.-106.degree. C. MS (FAB) m/z:. . .
DETD . . . dry tetrahydrofuran at room temperature. After stirring at room temperature for 36 hours, the reaction mixture is chromatographed on silica **gel** eluting with 50% acetone in hexanes to afford the title compound.
DETD . . . mL of methanol. The reaction mixture was stirred at room temperature under nitrogen overnight and then poured onto a silica **gel** column and eluted with 35% acetone in hexanes to give partially purified material. This material was rechromatographed on silica **gel** eluting with 25% acetone in hexanes to afford 462 mg. This material was rechromatographed on silica **gel** eluting

with 1:1 ethyl acetate-hexane to afford 108 mg of pure title compound, m.p. 102.degree.-106.degree. C. MS (FAB) m/z: M+K=966.

DETD . . . and water. The organic phase was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by silica **gel** column chromatography eluting with 25% acetone in hexanes to afford partially purified compound which was rechromatographed on silica **gel** eluting with 2% isopropanol in methylene chloride to give pure title compound (270.7 mg). m.p. 94.degree.-98.degree. C. MS (FAB) m/z:. . .

DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . was washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product was purified by silica **gel** chromatography eluting with 40% acetone in hexanes to afford 0.41 g of the title compound. MS (FAB) m/z: M+K=994.

DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . g) and DDQ (2 equivalent) is stirred in wet dichloromethane at room temperature overnight. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . Example 58 (1 g) in chloroform is stirred at 50.degree.-60.degree. C. for 4 hours. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . minutes. The reaction was then warmed to ambient temperature and stirred for 5 days. The mixture was adsorbed onto silica **gel** by dilution of the mixture with CH.sub.2 Cl.sub.2 (5 mL) followed by addition of silica **gel** (70-230 mesh, 60 A, 5 mL) and solvent evaporation. The adsorbed silica bed was placed on a fresh pad of. . .

DETD . . . of Example 99 is treated with dichlorodicyanobenzoquinone in warm benzene. The mixture is concentrated and purified by chromatography on silica **gel** to provide pure title compound.

DETD . . . (257 mg, 1.88 mmol) is added, and stirring is continued overnight. The reaction mixture is purified by chromatography on silica **gel** to provide the title compound.

DETD . . . SO.sub.4), filtered, and the solvent removed in vacuo to give crude title compound with is purified by chromatography on silica **gel**.

DETD . . . stirred at room temperature for 5 days, volatiles are removed in vacuo. The product is isolated by chromatography on silica **gel** as described in Example 98.

DETD . . . 172 and then treated with benzoic acid instead of morpholine, whereupon the mixture is heated. Purification by chromatography on silica **gel** provides the title compound.

DETD . . . of the ice and is stirred for 5 days. The reaction is diluted in diethyl ether and poured onto silica **gel** (70-230 mesh, 20 mL) and allowed to air dry. The adsorbed silica is layered on fresh silica (70-230 mesh, 100. . .

DETD . . . the ice and is stirred for 5 days. The reaction is diluted in diethyl ether (25 mL), poured onto silica **gel** (70-230 mesh, 40 mL) and allowed to air dry. The adsorbed silica is layered on fresh silica (70-230 mesh, 200. . .

DETD . . . The mixture is warmed to ambient temperature and stirred for 5 days. Purification of the mixture by chromatography on silica **gel** provides the title product.

DETD . . . temperature over 8 hours and is stirred for an additional 5

hours. Purification of the mixture by chromatography on silica **gel** provides title product.

DETD . . . of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise). . .

DETD . . . a pharmaceutically acceptable carrier or excipient, which may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, **ointments**, drops or transdermal patch), bucally, or as an oral or nasal spray. The phrase "pharmaceutically acceptable carrier" means a non-toxic. . .

DETD **Topical** administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for **topical** administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized. . .

DETD A further form of **topical** administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as automimmue diseases, allergic. . . aqueous humor, vitreous humor, cornea, iris/cilary, lens, choroid/retina and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an **ointment**, vegetable oil or an encapsulating material.

L9 ANSWER 27 OF 68 USPATFULL

AB A class of 2,6-diarylpyridazinones of general structural formula I have been identified that exhibit exhibit immunosuppressant activity with human T-lymphocytes, and are useful as an immunosuppressants. ##STR1## or a pharmaceutically acceptable salt, hydrate or crystal form thereof

AN 97:86612 USPATFULL

TI 2,6-diaryl pyridazinones with immunosuppressant activity

IN Bochis, Richard J., East Brunswick, NJ, United States
Kotliar, Andrew, Somerset, NJ, United States
Parsons, William H., Belle Mead, NJ, United States
Rupprecht, Kathleen, Cranford, NJ, United States

PA Merck & Co. Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5670504 19970923 <--

AI US 1995-392588 19950223 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Daus, Donald G.

LREP Camara, Valerie J., Daniel, Mark R.

CLMN Number of Claims: 6

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 3200

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5670504 19970923 <--

SUMM . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, Graves ophthalmopathy and asthma.

DETD . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous

Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne, Alopecia areata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .

DETD . . . parenteral applications. The active ingredient may be compounded, for example, with the usual non- toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .

DETD . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of: **azathioprine**, brequinar sodium, deoxyspergualin, mizadbine, mycophenolic acid morpholino ester, cyclosporin, FK-506 and rapamycin.

DETD . . . residue was dissolved in n-hexane:ethyl acetate (2:1) (approximately 400 ml) and the solution was passed over 1000 g of silica **gel**. Elution with n-hexane:ethyl acetate (3:1) yielded 11.66 g of 1-chloro-1-[(4-methoxyphenyl)hydrazono]-2-propanone, mp 114.degree.-116.degree. C. (hexane). A more preferred process for the. . .

DETD . . . sulfate and evaporated in vacuo. The residue was dissolved in 6:1 hexane:ethyl acetate and chromatographed over 1000 g of silica **gel**. Elution with hexane ethyl acetate (6:1) yielded 16.9 g 4-t-butoxynitrobenzene as an oil.

DETD . . . The solvent was removed in vacuo to yield the crude product. The residue was chromatographed of 100 g of silica **gel** to yield 355 mg of 1-[(4-trifuoromethoxyphenyl)thio]-1-[(4-methoxyphenyl)hydrazono]-2-propanone as a red oil.

DETD . . . was heated at 300.degree. C. for 1 hour. The reaction mixture was cooled and passed over 50 g of silica **gel**. Elution with n-hexane:ethyl acetate (2: 1) yielded 345 mg of S-4-methylthiophenyl dimethylthiocarbamate, mp 98.degree.-100.degree. C.

DETD . . . was removed in vacuo to yield 2.9 g of crude product. The residue was chromatographed over 200 g of silica **gel**. Elution with n-hexane:ethyl acetate (2:1) yielded 2.75 g of S-4-(methylsulfonylphenyl dimethylthiocarbamate

DETD . . . over magnesium sulfate and evaporated in vacuo to yield crude product. The residue was chromatograped over 100 g of silica **gel** . Elution with methylene chloride yielded 1.3 g of 4-methylsulfonylthiophenol, mp 54.degree.-58.degree. C.

DETD . . . mmol) was heated at 300.degree. C. for 3 hr. The reaction mixture was cooled chromatographed over 100 g of silica **gel**. Elution with n-hexane:ethyl acetate (4:1) yielded 616 mg of rearranged product.

DETD . . . and evaporated in vacuo. The residue was dissolved in 4:1 hexane: ethyl acetate and chromatographed over 100 g of silica **gel**. Elution with 4:1 hexane:ethyl acetate yielded 221 mg of semi pure 1-[(4-isopropylphenyl)thio]-1-[(4-methoxyphenyl)hydrazono]-2-propanone, as a red oil.

DETD . . . was heated at reflux for four hours. The solvent was removed in vacuo and the residue was chromatographed over silica **gel**. Elution with methylene chloride:isopropanol (100:2) yielded purified product, mp 138.degree.-145.degree. C.

DETD . . . of 1-chloro-1-[(4-methoxyphenyl)hydrazono]-2-propanone. The purity of the product was sufficient for further utilization. Further purification was accomplished by chromatography over silica **gel** and elution with elution with n-hexane:ethyl acetate (3:1) to yield

1-chloro-1 -[(4-methoxyphenyl)hydrazono]-2-propanone, mp
114.degree.-116.degree. C. (hexane).
DETD . . . (GIBO). Cells were pelleted by centrifugation at 1500 rpm for
8
minutes. Contaminating red cells were removed by treating the
pellet with ammonium chloride lysing buffer (GIBO) for 2 minutes
at 4.degree. C. Cold medium was added and cells were again. . .
CLM What is claimed is:
6. The pharmaceutical formulation of claim 5, comprising in addition,
an
antiproliferative agent selected from the group consisting of:
azathioprine, brequinar sodium, deoxyspergualin, mizaribine,
mycophenolic acid morpholino ester, cyclosporin, FK-506 and rapamycin.

L9 ANSWER 28 OF 68 USPATFULL
AB Compounds and methods for use in immunosuppressive and
anti-inflammatory
treatment are described. The compounds are triptolide analogs with
improved water solubility and low toxicity.
AN 97:78602 USPATFULL
TI Immunosuppressive compounds and methods
IN Qi, You Mao, Los Altos, CA, United States
Musser, John H., San Carlos, CA, United States
PA Pharmagenesis, Inc., Palo Alto, CA, United States (U.S. corporation)
PI US 5663335 19970902 <--
AI US 1996-609277 19960301 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Reamer, James H.
LREP Powers, Vincent M., Gorthey, LeeAnn
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1057
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5663335 19970902 <--
SUMM . . . drugs and low dose corticosteroids); disease-modifying
antirheumatic drugs, known as "DMARDs" (antimalarials, gold salts,
penicillamine, and sulfasalazine) and immunosuppressive agents (
azathioprine, chlorambucil, high dose corticosteroids,
cyclophosphamide, methotrexate, nitrogen mustard, 6-
mercaptopurine, vincristine, hydroxyurea, and cyclosporin A).
None of the available drugs are completely effective, and most are
limited by severe toxicity.
SUMM Another obstacle in transplantation, which has limited bone marrow
transplants (BMT) in particular, is graft-versus-host disease (
GVHD). **GVHD** is a condition in which transplanted
marrow cells attack the recipient's cells (Thomas, 1975; Storb, 1984).
Many BMT patients receiving HLA-identical marrow that tests negative in
the mixed lymphocyte reaction (MLR) still develop **GVHD**,
presumably because of a disparity between the recipient and donor at
polymorphic non-HLA determinants. A large proportion of **GVHD**
-afflicted individuals die as a result of **GVHD** (Weiden, et
al., 1980).
SUMM . . . for preventing transplant rejection include corticosteroids,
antimetabolite drugs that reduce lymphocyte proliferation by inhibiting
DNA and RNA synthesis such as **azathioprine**, immunosuppressive
drugs such as cyclosporin A, which specifically inhibits T cell
activation, and specific antibodies directed against T lymphocytes or.

. .
DETD . . . or liquid dosage forms, such as, for example, tablets, pills, capsules, powders, sustained-release formulations, solutions, suspensions, emulsions, suppositories, retention enemas, **creams**, **ointments**, lotions, aerosols or the like, preferably in unit dosage forms suitable for simple administration of precise dosages.
DETD . . . unable to swallow, or oral absorption is otherwise impaired, the preferred systemic route of administration will be parenteral, intranasal, or **topical**.
DETD . . . is worked up by filtering off the dicyclohexylurea, removing the solvent by evaporation, and chromatographing the obtained solid on silica **gel**.
DETD . . . The dicyclohexylurea is filtered off, and the solvent is removed by evaporation. The crude product is then chromatographed on silica **gel**.
DETD . . . is worked up by filtering off the dicyclohexylurea, removing the solvent by evaporation, and chromatographing the obtained solid on silica **gel**.

L9 ANSWER 29 OF 68 USPATFULL
AB Methods of treatment for inflammatory and autoimmune dermatoses which comprises **topical** and/or systemic administration of a therapeutically-effective amount of thalidomide alone or in combination with other dermatological agents.
AN 97:68480 USPATFULL
TI Treatment of inflammatory and/or autoimmune dermatoses with thalidomide alone or in combination with other agents
IN Andrulis, Jr., Peter J., Bethesda, MD, United States
Drulak, Murray W., Gaithersburg, MD, United States
PA Andrulis Pharmaceuticals, Beltsville, MD, United States (U.S. corporation)
PI US 5654312 19970805 <--
AI US 1995-475426 19950607 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Nutter, Nathan M.
LREP Angres, Isaac
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 925
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5654312 19970805 <--
AB Methods of treatment for inflammatory and autoimmune dermatoses which comprises **topical** and/or systemic administration of a therapeutically-effective amount of thalidomide alone or in combination with other dermatological agents.
SUMM . . . 1982) were the first to use thalidomide to treat 22 patients with Behcet's syndrome who had deep and persistent oral **aphthae**. Patients were initially administered 400 mg per day of thalidomide for
five days followed by 200 mg per day for 15 to 60 days. This regimen resulted in rapid and complete healing of **aphthae**. Torras et al. (Arch. Dermatol, 118:875, 1982) found that there was complete healing of giant **aphthae** in eight of nine Behcet's patients treated with 100 mg per day of thalidomide for 10 days. Jorizzo et al.. . . treatment time of up to 65 months. Concomitant treatment in this patient group included 10 patients on prednisone, 3 on **azathioprine** and 1 patient on cyclosporin. Mucosal lesions healed in all patients. Moulin et al. (Ann. Dermatol Venereol, 110:611,

1983) used. . .

SUMM . . . successfully used to treat a limited number of dermatoses that may have an autoimmune and/or inflammatory component associated with them. **Topical** application of thalidomide is a useful therapeutic approach for disease states with an autoimmune and/or inflammatory basis. Furthermore, thalidomide may. . .

SUMM . . . be used topically to treat dermatoses with an autoimmune and/or inflammatory component associated with them, such as, for example, using **creams**, **ointments** or lotions or in combination with other therapies.

SUMM . . . have not been clearly defined at the molecular level, thalidomide has been used to treat the following immunologically-based diseases: acute **aphthous** ulcers (Jenkins et al., Lancet, 2:1424-6, 1984; Grinspan, J. Amer. Acad. Dermatol, 12:85-90, 1985; Revuz et al., Arch. Dermatol, 126:923-7,. . .

SUMM The present invention is directed to a method for the **topical** and/or systemic treatment of inflammatory and autoimmune dermatoses in a mammal which comprises applying and/or administering to said mammal a. . .

SUMM The instant invention is also directed to a method for the **topical** and/or systemic treatment of inflammatory and autoimmune dermatoses in a mammal which comprises applying to involved areas of the body. . .

SUMM . . . as acute, chronic and physical urticarias, for example solar, cholinergic, pressure and cold urticarias. Atopic dermatitis; Mast Cell Disease, Bullous **Pemphigoid**; **Pemphigus Vulgaris**; necrotizing vasculitis; lupus erythematosus (discold and systemic); dermatitis herpetiformis.

SUMM (r) Diseases of Mucous Membranes: such as **aphthous** ulcers.

SUMM There are two general forms of treatment for dermatoses: (1) physical therapies (2) chemical therapies including **topical** and systemic administration of agents.

SUMM **Topical** Medications

SUMM . . . Antiseptics, which inhibit and/or destroy fungi and/or bacteria, such as 3% Vioform, 3-10% ammoniated mercury, antifungal agents such as Whitfields **ointment**, antibiotics such as 3% terramycin, 0.5% neomycin, 0.1% garamycin and 3% aureomycin.

SUMM (g) Antiparasitics, which inhibit or destroy infestations by parasites, such as Kewell **cream** for scabies and pediculosis and Eurax lotion for scabies.

SUMM **Topical** medications are delivered to the effected site including but not exclusive to the following methods:

SUMM (k) **Creams** and **Ointments**: such as those with water washable **cream** bases, those with **ointment** bases, antifungal antibiotics, corticosteroids, antipruitic **creams** and fluorinated corticosteroids.

SUMM Although many dermatoses can be adequately treated with physical therapies or **topical** medications in certain instances systemic chemotherapy is superior. The following list of chemotherapeutic agents used systemically to treat dermatoses is. . .

SUMM In treating Kaposi's Sarcoma, an **ointment** containing 10% by weight of thalidomide is applied to the lesion. In an alternative embodiment, Kaposi's Sarcoma is treated concurrently by **topical** and oral treatment. For example, a patient presenting with Kaposi's Sarcoma is treated daily for two to four weeks with a dosage amount of

50 mg of thalidomide a day while an **ointment** containing 10% by weight thalidomide is applied to the lesion three times a day for two to four weeks.

SUMM When used alone, the topically effective amounts of thalidomide are typically 5 to 15% by weight in an **ointment** and is applied one to three times a day for a period of time to induce regression of the dermatoses.

SUMM Under certain circumstances, it is desirable to administer thalidomide therapy simultaneously with other dermatological active agents. For example, a **cream** containing 5% by weight of thalidomide can be administered three times a day while the patient is being given a **topical** treatment with 1% hydrocortisone. Concurrent administration of oral thalidomide with **topical** thalidomide is also a desirable therapeutic goal.

SUMM For humans, typically-effective amounts of thalidomide for use in the **topical** dosage forms compositions of the present invention, range from 5-15% by weight active, however, greater amounts may be employed if. . .

SUMM . . . in combination with other compounds. Preferably the compounds of the present invention are administered orally, intramuscularly, topically, subcutaneously, or intravenously. **Topical** administration is particularly preferred.

SUMM . . . the compounds of the present invention, pharmaceutically-acceptable carriers can be either solid or liquid. Solid form preparation include powders, lotions, **creams**, **ointments**, tablets, pills, capsules, cachets, suppositories, and dispensable granules. A solid carrier can be one or more substances which may also. . .

SUMM . . . with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and **lozenges** are included. Tablets, powders, capsules, pills cachets, and **lozenges** can be used as solid dosage forms suitable for oral administration.

SUMM Liquid form preparations such as lotions or **creams** include solutions, suspensions, and emulsions, for example, water or DMSO/propylene glycol solutions. For parenteral injection, liquid preparations can be formulated. . .

SUMM Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for **topical** or systemic administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, addition to the active component,. . .

SUMM . . . capsules, lotions and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or **lozenge** itself, or it can be the appropriate number of any of these in packaged form.

SUMM . . . it is possible to use the previously mentioned substances and spreadable or liquid hydrocarbons such as Vaseline or paraffin or **gels** of alkanes and polyethylene, fats and oils of plant or animal origin, which may in part also be hydrated, or. . .

DETD A **topical ointment** containing thalidomide is prepared as follows:

DETD A **gel** is made as follows:

DETD **Ointment** containing thalidomide:

DETD . . . induction of a granular layer and orthokeratosis in areas of scale between the hinges of the tail epidermis. Typically, a **topical ointment** is examined histologically. An additional model is provided by grafting psoriatic human applied daily for seven consecutive days, then the. . .

DETD Twenty patients suffering from psoriasis are to be treated with a **cream** containing 8% by weight of thalidomide.

DETD . . . with an appropriate placebo and a commercially available product. This commercially available product should be designated the "control", whereas the **cream** containing 8% by weight of thalidomide should be the "test" **cream**.

DETD . . . should be carried out by a consultant dermatologist as a double blind trial, each patient using the test or control **creams** twice daily, the **cream** being applied to the area of the arms affected by this skin disorder.

DETD . . . four weeks, after which the results should be assessed by the consultant dermatologist. It will be shown that the test **cream** produces an improvement in the condition of the skin of each patient, as compared with the placebo **cream**. Furthermore, the "test" **cream** will be more cosmetically acceptable than the control **cream**, and will result in fewer complaints from the subjects being treated.

DETD Forty patients suffering from moderate acne are to be treated with a **cream** containing 5% by weight thalidomide.

DETD Upon completion of the treatment period, the areas treated with the 5% by weight thalidomide **cream** will exhibit a clinically significant decrease in the severity of acne as compared to placebo treatment. Furthermore, the thalidomide-treated subjects. . .

DETD Two patients exhibiting leg lesions and diagnosed as being Kaposi's sarcoma are to be treated with a **cream** containing 10% by weight thalidomide.

DETD Upon completion of the treatment period, the area treated with the 10% by weight **cream** will exhibit a clinical improvement and will exhibit less severe side effects.

DETD Following the protocol of Example 13, two patients are treated except that concurrently with **topical** administration they are orally treated with 50 mg/day of thalidomide for the duration of the **topical** treatment.

L9 ANSWER 30 OF 68 USPATFULL

AB The invention provides purified ARAg polypeptides, antibodies against ARAg polypeptides and nucleic acids encoding ARAg polypeptides. Also provided are methods of diagnosis and treatment using the same. ARAg polypeptides are typically present on the surface of alloantigen-activated CD8.sup.+ T-cells, monocytes, granulocytes and peripheral dendritic cells, and substantially absent on resting T-cells, mitogen-activated CD8.sup.+ T-cells, B-cells, erythroid cell lines, myelomonocitic cell lines, EBV-LCL cell lines and fibroblastoid cell lines. An exemplary ARAg polypeptide, termed ARAg-h-1, has a signal sequence, seven variable-type immunoglobulin-like domains, a transmembrane domain and an intracellular domain.

AN 97:59308 USPATFULL

TI Alloreaction-associated antigen (ARAG): a novel member of the immunoglobulin gene superfamily

IN Ruegg, Curtis L., San Carlos, CA, United States
Rivas, Alberto, Palo Alto, CA, United States
Laus, Reiner, Belmont, CA, United States
Engleman, Edgar G., Atherton, CA, United States

PA The Board of Trustees of Leeland Stanford Jr. Univ., Palo Alto, CA, United States (U.S. corporation)

PI US 5646251 19970708 <--

AI US 1995-497025 19950630 (8)

RLI Continuation-in-part of Ser. No. US 1993-149212, filed on 5 Nov 1993, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Walsh, Stephen G.; Assistant Examiner: Kemmerer, Elizabeth C.
LREP Townsend and Townsend and Crew LLP
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 15 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 2085
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5646251 19970708 <--
DETD . . . the .alpha.3 domain, T-cell antigens (e.g., OKT4 and OKT3), antithymocyte globulin, as well as chemotherapeutic agents such as cyclosporine, glucocorticoids, **azathioprine**, prednisone can be used in conjunction with the therapeutic agents of the present invention.
DETD . . . for the therapeutic agents of the present invention is in modulating the immune response involved in "graft versus host" disease (**GVHD**). **GVHD** is a potentially fatal disease that occurs when immunologically competent cells are transferred to an allogeneic recipient. In this situation,. . . recipient. Tissues of the skin, gut epithelia and liver are frequent targets and may be destroyed during the course of **GVHD**. The disease presents an especially severe problem when immune tissue is being transplanted, such as in bond marrow transplantation; but less severe **GVHD** has also been reported in other cases as well, including heart and liver transplants. The therapeutic agents of the present. . .
DETD . . . (pH 7.5), 250 mM NaCl, 1% Triton X-100) containing 0.25M sucrose and microcentrifuging for 3 min at 13,000 g. The **pellet** was washed with 1 ml lysis buffer supplemented with 2M urea and incubated for 2 min at room temperature. After. . . 70 .mu.l SDS-PAGE buffer containing of 5% 2-mercaptoethanol to elute bound radiolabeled protein. Samples were analyzed on 6% SDS PAGE **gels**. **Gels** were stained with Coomassie blue and dried. The radioactive bands were visualized by fluorography at -70.degree. C.
DETD . . . a molecular weight of about 135 kDa. A minor band of about 218 kDa appeared to be co-precipitated in some **gels** but disappeared on extensive washing, and is probably artifactual. Similar results were obtained when the above procedure was repeated using. . .
DETD . . . native ARAg mRNA transcripts was determined by purifying poly(A).sup.+ mRNA from fresh human monocytes, fractionating mRNA on a denaturing agarose **gel**, transferring the mRNA to a nylon membrane and hybridizing with a .sup.32 P-labeled DNA probe spanning bases 1-2038 of the. . .

L9 ANSWER 31 OF 68 USPATFULL
AB A method for the treatment of a cutaneous, ocular, or mucosal pathological condition which is associated with immune response in a human or other mammal, that includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.
AN 97:52005 USPATFULL

TI **Topical** application of spiperone or derivatives thereof for treatment of pathological conditions associated with immune responses
IN Sharpe, Richard J., Gloucester, MA, United States
Arndt, Kenneth A., Newton Centre, MA, United States
Galli, Stephen J., Winchester, MA, United States
PA Beth Israel Deaconess Medical Center, Inc., Boston, MA, United States (U.S. corporation)
PI US 5639758 19970617 <--
AI US 1993-120218 19930913 (8)
RLI Continuation of Ser. No. US 1992-831429, filed on 5 Feb 1992, now patented, Pat. No. US 5244902 which is a continuation-in-part of Ser. No. US 1990-494744, filed on 16 Mar 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-396523, filed on 21 Aug 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Dees, JoseG.; Assistant Examiner: Cebulak, Mary C.
LREP Kilpatrick & Cody, L.L.P., Meredith, Roy D.
CLMN Number of Claims: 3
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 891
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI **Topical** application of spiperone or derivatives thereof for treatment of pathological conditions associated with immune responses
PI US 5639758 19970617 <--
AB . . . cutaneous, ocular, or mucosal pathological condition which is associated with immune response in a human or other mammal, that includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.
SUMM This invention is in the area of the **topical** treatment of cutaneous, ocular, and mucosal hypersensitivity and hyperproliferative conditions induced by or associated with an immune response, that includes. . .
SUMM . . . Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, lichen planus, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions.. . .
SUMM . . . agents with partial utility for treating some of the above conditions include psoralen plus ultraviolet A (PUVA), cyclosporin A, or **azathioprine**, but the risk-to-benefit ratios for these agents is unfavorable for most of the conditions described above.
SUMM U.S. Pat. No. 4,874,766 assigned to Janssen Pharmaceutica N.V. discloses a method for promoting wound-healing by **topical** administration of a serotonin-antagonist compound, including spiperone and its derivatives. Wound healing is a reparative process by which several types. . .
SUMM It is an object of the present invention to present a method for the **topical** treatment of cutaneous, mucosal and ocular pathology associated with immune responses.
SUMM It is yet another object of the present invention to present a method for the **topical** treatment of cutaneous, mucosal, or ocular

hypersensitivity and epithelial hyperproliferation.

SUMM It is yet another object of the invention to present a method for the **topical** treatment of cutaneous, mucosal or ocular scarring.

SUMM . . . ocular, or mucosal condition in a human or other mammal resulting from pathology associated with an immune or other mammal includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.

SUMM . . . exhibits a strong immunosuppressive activity when applied topically. The parent spiperone is used herein as the model of an active **topical** immunosuppressant. Spiperone derivatives are measured against this model, and are considered to be immunosuppressants if they suppress the leukocyte infiltration. . .

SUMM . . . administered topically in a suitable carrier to effectively immunosuppress the patient at the site of application. Because the application is **topical**, i.e., local, immunosuppression is achieved without producing systemic effects, most notably, the significant neuroleptic effect that is associated with the. . .

SUMM Spiperone and its active derivatives are useful as **topical** agents in treating contact dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions. The novel. . .

DRWD . . . hypersensitivity reactions. These data (mean .+-.SEM) are from the same mice whose ear thickness measurements are presented in FIG. 5. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**p<0.01).

DRWD FIGS. 8a,b,c--Effect of **topical** treatment with spiperone on leukocyte infiltration associated with oxazolone-induced contact hypersensitivity reactions. These data (mean .+-.SEM) are from the same.

. . are presented in FIGS. 7a,b,c. Biopsies were performed 24 hours (a, b) or 46 hours (c) after application of oxazolone. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**=p<0.01). In FIG. 8a, the slight. . .

DRWD FIG. 10--Effect of **topical** treatment with spiperone on leukocyte infiltration associated with DNFB-induced contact hypersensitivity reactions. These data (mean .+-.SEM) are from the same mice whose ear thickness measurements are presented in FIG. 9. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**p<0.01). The slight effect of treatment. . .

DETD Mammals, and specifically humans, suffering from pathogenic cutaneous, ocular, or mucosal immune responses can be treated by **topical** administration to the patient of an effective amount of the spiperone derivative or its salt in the presence of a. . .

DETD Solutions or suspensions for **topical** application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,. . .

DETD Suitable vehicles or carriers for **topical** application are known, and include lotions, suspensions, **ointments**,

creams, **gels**, tinctures, sprays, powders, pastes, slow-release transdermal patches, aerosols for asthma, suppositories for application to rectal, vaginal, nasal or oral mucosa,. . .

DETD Thickening agents, emollients, and stabilizers can be used to prepare **topical** compositions. Examples of thickening agents include petrolatum, beeswax, xanthan gum, or polyethylene glycol, humectants such as sorbitol, emollients such as mineral oil, lanolin and its derivatives, or squalene. A number of solutions and **ointments** are commercially available, especially for ophthalmic and dermatologic applications.

DETD Natural or artificial flavorings or sweeteners can be added to enhance the taste of **topical** preparations applied for local effect to mucosal surfaces. Inert dyes or colors can be added, particularly in the case of. . .

DETD . . . potential irritancy or neuropharmacological effects of the composition. See, in general, Arndt, K. A., P. V. Mendenhall, "The Pharmacology of **Topical** Therapy", Dermatology in General Medicine, 1987; T. B. Fitzpatrick, A. Z. Eisen, K. Wolff, I. M. Freedberg and K. F.. . .

DETD Spiperone and spiperone derivatives are capable of suppressing the immune response in humans and other mammals on **topical** application. As such, the compounds, or therapeutic compositions thereof, are useful for the treatment of a myriad of immunological disorders. Pathogenic immune responses that can be treated by **topical** application of spiperone or spiperone derivatives include contact dermatitis, atopic dermatitis, eczematous dermatitis, drug eruptions, lichen planus, psoriasis, alopecia areata,. . . Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, cutaneous lupus erythematosus, scleroderma, allergic reactions secondary to arthropod bite reactions, **aphthous** ulcers, conjunctivitis, keratoconjunctivitis, iritis, asthma and allergic asthma, vaginitis, Crohn's disease, ulcerative colitis and proctitis. These compounds can also be. . .

DETD . . . ensues from the dry eye state. Spiperone or its active derivatives can be provided as an ophthalmic drop or ophthalmic **ointment** to humans or other mammals, including dogs and cats, in an effective amount in a suitable vehicle. This **topical** ophthalmic treatment can also serve to correct corneal and conjunctival disorders exacerbated by tear deficiency and KCS, such as corneal. . .

DETD . . . the tissue swelling and the leukocyte infiltration associated with the elicitation phase of contact hypersensitivity to either oxazolone or dinitrofluorobenzene. **Topical** treatment with spiperone also suppressed the sensitization phase of contact sensitivity. However, mice treated topically with spiperone, unlike those treated. . .

DETD **Topical** Spiperone Treatment--To test whether spiperone affected the sensitization phase of contact hypersensitivity, 50 .mu.l of 0.08% spiperone in propylene glycol. . .

DETD . . . infiltration at sites of hapten challenge than did vehicle-treated mice ($p<0.01$ for either comparison). These data show that treatment with **topical** spiperone can effectively inhibit the sensitization phase of cutaneous contact hypersensitivity.

DETD Effects of **Topical** Spiperone on Expression of Contact Hypersensitivity--For these experiments, both ears of each mouse were challenged for elicitation of contact hypersensitivity. . . skin) to both surfaces of the ears. The right ears of control mice were similarly

treated, but with vehicle alone. **Topical** administration of a 4.0% suspension of spiperone in absolute ethanol, propylene glycol, and olive oil one hour after hapten challenge. . .

DETD Although **topical** application of spiperone was extremely effective in diminishing both the tissue swelling and the leukocyte infiltration associated with contact hypersensitivity. . .

DETD To evaluate the effect of **topical** treatment with spiperone on contact hypersensitivity reactions elicited with a different hapten, the effect of **topical** treatment with a 0.5% suspension of spiperone on the contact hypersensitivity reactions elicited with DNFB was examined. **Topical** treatment with spiperone significantly diminished the tissue swelling associated with reactions to DNFB (by 45%, FIG. 9) and had an. . .

DETD Mice were sensitized to oxazolone as described in Example 1. Three days later, slow release indomethacin **pellets** (0.05 mg, 3 week release) were implanted subcutaneously under light ether anesthesia. The dose of indomethacin delivered by these **pellets** has been previously shown to completely block prostaglandin synthesis in mice, by Jun, D. D., et al., J. Invest. Dermatol.. . .

DETD . . . and variations of the present invention relating to methods for the treatment of pathology associated with immune responses that includes **topical** administration of an effective amount of spiperone or a spiperone derivative will be obvious to those skilled in the art. . .

CLM What is claimed is:
1. A **topical** pharmaceutical composition for the treatment of a cutaneous, ocular, or mucosal pathology associated with an immune response in a human. . .
2. A **topical** pharmaceutical composition for the treatment of a cutaneous, ocular, or mucosal pathology associated with an immune response in a human. . .
3. A **topical** pharmaceutical composition for the treatment of a cutaneous, ocular, or mucosal pathology associated with an immune response in a human. . .

L9 ANSWER 32 OF 68 USPATFULL

AB A method for the **topical** or systemic treatment of disorders mediated by proteases which result in skin or mucosal lesions, and in particular, pemphigus, cicatricial **pemphigoid**, bullous **pemphigoid**, lichen planus, and canker sores, is disclosed wherein the host is treated with an effective amount of N-acetyl ysteine or a derivative thereof, or its pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable diluent or carrier for systemic or **topical** delivery.

AN 97:49665 USPATFULL

TI Method for treating diseases mediated by proteases

IN Sharpe, Richard J., Gloucester, MA, United States
McAloon, Maureen H., Boston, MA, United States
Galli, Stephen J., Winchester, MA, United States
Arndt, Kenneth A., Newton Centre, MA, United States

PA Arcturus Pharmaceutical Corporation, Woburn, MA, United States (U.S. corporation)

PI US 5637616 19970610 <--

AI US 1993-131892 19931005 (8)

RLI Continuation-in-part of Ser. No. US 1993-79645, filed on 18 Jun 1993,

now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: O'Sullivan, Peter
LREP Kilpatrick & Cody, L.L.P.
CLMN Number of Claims: 48
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1049
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5637616 19970610 <--
AB A method for the **topical** or systemic treatment of disorders mediated by proteases which result in skin or mucosal lesions, and in particular, pemphigus, cicatricial **pemphigoid**, bullous **pemphigoid**, lichen planus, and canker sores, is disclosed wherein the host is treated with an effective amount of N-acetyl ysteine or a derivative thereof, or its pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable diluent or carrier for systemic or **topical** delivery.
SUMM This invention is a method for the treatment of diseases mediated by proteases that includes the **topical** or systemic administration of an effective amount of N-acetylcysteine or a derivative or salt thereof.
SUMM . . . membranes which have been found to be mediated by proteases. Examples of protease mediated disorders include lichen planus, canker sores (**aphthous** ulcers), and a number of bullous diseases, including but not limited to pemphigus, bullous **pemphigoid** and cicatricial **pemphigoid**.
SUMM . . . method of treating ulcerative lichen planus symptoms is with intra-lesional steroid injections, which is often repeated at frequent intervals. Potent **topical** steroids such as beta-methasone dipropionate and clobestol propionate are also be helpful, but the medication must be applied very frequently (every hour or so). **Topical** tretinoin, cyclosporine, and systemic antifungal agents, such as griseofulvin, have been reported to be somewhat effective in treating severely symptomatic. . . oral lichen planus. No large, well designed studies, however, have proven the efficacy of these therapies. The use of potent **topical** steroids, particularly on mucosal surfaces, can result in dangerous side effects.
SUMM . . . or infection if serious bullous disease is not adequately treated. Bullous diseases include, but are not limited to, pemphigus, bullous **pemphigoid**, and cicatricial **pemphigoid**.
These three typical examples of bullous conditions are briefly described below.
SUMM . . . 56-60). Pemphigus can be further categorized by the specific site of the blisters in the various layers of the epidermis. **Pemphigus vulgaris** and Pemphigus vegetans exhibit blisters above the basal layer of the skin (i.e., the first layer of keratinocytes in the. . .
SUMM **Pemphigus vulgaris** can affect all age groups. Lesions usually occur in the mouth, as well as on the chest, scalp, periumbilical, and. . . the disease can involve the oropharynx and other mucosal surfaces, sometimes extending into the esophagus and cardia of the stomach. **Pemphigus vulgaris** is characterized by intra-epidermal blister formations due to acantholysis (i.e., loss of intercellular adhesions) in the superbasilar epidermis and intact. . .

SUMM . . . patients must be closely monitored for adrenocorticoid side effects. It has also been reported that immunosuppressive agents such as cyclophosphamide, **azathioprine**, methotrexate and cyclosporine-A, or a combination of immunosuppressive agents with high doses of prednisone may be useful in the symptomatic. . .

SUMM Bullous **Pemphigoid**

SUMM Bullous **pemphigoid** is the most common bullous disease of the skin. It is more prevalent in elderly patients than in younger patients.. . .

SUMM As with pemphigus, treatments for the various forms of bullous **pemphigoid** include systemic glucocorticosteroids. Often treatment will include an immuno-suppressive agent in addition to the steroids. Intra-lesional steroids may be beneficial in preventing scarring and may be used to treat mucous membrane disease. **Topical** treatments including steroid **creams** and Burows' solution baths are used to prevent secondary infection and scarring.

SUMM Cicatricial **Pemphigoid**

SUMM Cicatricial **pemphigoid**, also called benign mucous membrane **pemphigoid** or ocular **pemphigoid**, is an uncommon chronic subepidermal bullous dermatosis which involves primarily the mucous membranes (Baden, L. A., Manual of Clinical Problems. . .

SUMM . . . Austen, Dermatology in General Medicine, 1987, Vol. 1, McGraw-Hill, Inc., New York, pp. 582-584). As with pemphigus, treatment of cicatricial **pemphigoid** often requires high doses of systemic corticosteroids and immunosuppressive agents. Because of the scarring associated with cicatricial **pemphigoid**, long term systemic steroids have been used in these patients despite the side effects. Cyclophosphamide, methotrexate, dapsone and **azathioprine** have been beneficial to some patients, while others have shown little improvement with these agents. **Topical** and intra-lesional steroids seem to be less effective in cicatricial **pemphigoid** than in oral lichen planus.

SUMM A common feature of lichen planus, pemphigus, bullous **pemphigoid** , cicatricial **pemphigoid** and lichen planus is the role of proteases in their pathogenesis. For example, in one study, cytotoxic proteases were identified in the blister fluid of pemphigus and **pemphigoid** patients (Grando, Glukhenky, Drannik, Kostromin and Chernyavsky, Int. J. Tissue React. 1989, Vol. 11, pp. 195-201). Similar observations have been. . .

SUMM Canker Sores (**Aphthous** Ulcers)

SUMM **Aphthous** ulcers are inflammatory lesions of unknown etiology that can effect any mucosal surface, but occur most often in the mouth. . . the actions of a host of soluble mediators such as proteases and tumor necrosis factor. Current treatments include hygienic measures, **topical** anesthetics and various unproven therapies such as oral suspensions of tetracyclines and systemic and **topical** corticosteroids. Patients are frequently instructed to avoid trauma to the oral cavity (such as sharp bread crusts or hard toothbrushes). .

SUMM . . . of the seriousness of the symptoms associated with the disorders described above, there clearly remains a need for effective, safe **topical** and systemic methods for their treatment.

SUMM Therefore, it is an object of the present invention to provide a method for the **topical** treatment of disorders mediated by proteases.

SUMM A method for the **topical** or systemic treatment of disorders mediated by proteases that cause skin or mucosal lesions, and in particular, pemphigus, cicatricial **pemphigoid**, bullous **pemphigoid**, lichen planus, and canker sores (**aphthous**

ulcers), is disclosed wherein the host is treated with an effective amount of N-acetylcysteine ("NAC") or a derivative thereof, or its pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable diluent or carrier for systemic or **topical** delivery. The active compound or its derivative is administered for a sufficient time period to alleviate the undesired symptoms and. . .

SUMM . . . example, ocular, vaginal, nasal, or oral membranes) can be treated with an effective amount of N-acetylcysteine in a carrier for **topical** delivery. The active compound is administered in an effective dosage range to cause suppression of the symptoms. In one embodiment,. . . In another embodiment, an effective amount of N-acetylcysteine or its derivative or salt is applied to the lesion in a **cream**, **gel**, **ointment**, diluent, foam or paste, from one to several times a day.

SUMM . . . events which result in pathological tissue injury and thus should assist in accelerating the healing of painful lesions associated with **aphthous** ulcers and preventing the formation of new lesions.

DETD It has been discovered that disorders mediated by proteases can be treated by the **topical** or systemic administration of an effective amount of N-acetylcysteine, or a derivative thereof, or a pharmaceutically acceptable salt of N-acetylcysteine or a derivative thereof, optionally in a pharmaceutical carrier for **topical** or systemic delivery.

DETD . . . and Stockley, Biol. Chem. Hoppe Seyler 1986, Vol. 367, pp. 177-82). Given the complexity of disorders such as pemphigus, cicatricial **pemphigoid**, bullous **pemphigoid**, lichen planus, and canker sores, one could not predict from this report whether NAC would be an effective treatment in. . .

DETD . . . active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid, **cream**, **gel** or solid form.

DETD N-acetylcysteine or its derivative or salt is preferably applied in the form of a **topical** composition. The composition can be formulated in a variety of ways known to those skilled in the art, for example,. . . such as a solution or a suspension in an aqueous or oily medium; or a semi-liquid formulations such as a **cream**, jelly, paste, **ointment**, or salve. In one embodiment, the compound is applied in the form of a solution, **gel**, **ointment**, **cream**, lotion or foam, in a 1-100%, for example a 10-20% by weight, aqueous solution. Acetylcysteine is currently available in 10. . .

DETD Solutions or suspensions used for parenteral, intra-dermal, subcutaneous, or **topical** application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,. . .

DETD . . . of drugs into the skin. For other examples, see, in general, Arndt, K. A., Mendenhall, P. V., "The Pharmacology of **Topical** Therapy", Dermatology in General Medicine, 1987; Fitzpatrick, Eisen, Wolff, Freeberg, Austen, eds., 3d ed., McGraw Hill, Inc., New York, pp..

DETD . . . a host a therapeutically effective amount of the drug without causing serious toxic effects in the patient treated. A typical **topical** dosage will range from 1 to 30 weight percent in a suitable carrier. A preferred systemic dose of the active. . .

DETD Natural or artificial flavorings or sweeteners can be used to enhance

the taste and odor of **topical** preparations applied for local effect to mucosal surfaces. Inert dyes or colors can also be added, particularly for compositions designed. . .

DETD . . . also monitored in the animals' serum, to confirm transfer of the pemphigus antibodies. One group of mice is treated with **topical** administration of the test compound and monitored for disease improvement by sampling the skin and assessing its appearance by histology. . .

DETD . . . 4.degree. C. for 2 hours in 0.01M sodium monophosphate, pH 7.0 and centrifuged at 750 g for 10 min. The **pellet** is extracted with 2M potassium thiocyanate (KSCN) with 0.01% Triton X-100 4.degree. C. for 2 hours. The extracts are centrifuged. . .

DETD The effectiveness of treatment of patients with oral lesions resulting from lichen planus, bullous **pemphigoid**, cicatricial **pemphigoid**, pemphigus or canker sores (**aphthous** uclers) with NAC or its derivatives or salts thereof can be evaluated as described generally for treatment of lichen planus. . . Med. 1990, Vol. 323, pp. 290-4. For example, patients with symptomatic oral lichen planus are given either placebo or a **topical** N-acetylcysteine solution, **gel**, or **ointment** containing 1 to 50% NAC or other test compound. The solutions are swished for several minutes and expectorated or swallowed. . .

DETD . . . variations of the present invention relating to a method for the treatment of diseases mediated by proteases that includes the **topical** or systemic administration of an effective amount of N-acetylcysteine or a derivative or salt thereof will be obvious to those. . .

CLM What is claimed is:

. . . proteases in mammals that result in skin or mucosal lesions selected from the group consisting of lichen planus, canker sores (**aphthous** ulcers), and bullous diseases, comprising: topically applying to the skin or mucosal lesion an effective amount of N-acetylcysteine or a pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier to **topical** administration.

. . . proteases in mammals that result in skin or mucosal lesions selected from the group consisting of lichen planus, canker sores (**aphthous** ulcers), and bullous diseases, comprising: systemically administering to a mammal in need thereof an effective amount of N-acetylcysteine or a. . .

. . . proteases in mammals that result in skin or mucosal lesions selected from the group consisting of lichen planus, canker sores (**aphthous** ulcers), and bullous diseases, comprising: topically applying to the skin or mucosal lesion an effective amount of a derivative of. . . of an alkyl or aromatic dicarboxylic acid; or a pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier for **topical** administration.

. . . proteases in mammals that result in skin or mucosal lesions selected from the group consisting of lichen planus, canker sores (**aphthous** ulcers), and bullous diseases, comprising: systemically administering an effective amount of a derivative of N-acetylcysteine of the formula ##STR4## wherein. . .

7. The method of claim 1 wherein the compound is applied in the form of a 1 to 100% **topical** solution, **gel**, **ointment**

, **cream**, lotion or foam.

8. The method of claim 3 wherein the compound is applied in the form of a 1 to 100% **topical** solution, **gel**, **ointment** , **cream**, lotion or foam.

26. The method of claim 1 wherein the disease is bullous **pemphigoid**.

27. The method of claim 1 wherein the disease is cicatricial **pemphigoid**.

31. The method of claim 2 wherein the disease is bullous **pemphigoid**.

32. The method of claim 2 wherein the disease is cicatricial **pemphigoid**.

36. The method of claim 3 wherein the disease is bullous **pemphigoid**.

37. The method of claim 3 wherein the disease is cicatricial **pemphigoid**.

41. The method of claim 4 wherein the disease is bullous **pemphigoid**.

42. The method of claim 4 wherein the disease is cicatricial **pemphigoid**.

L9 ANSWER 33 OF 68 USPATFULL

AB A specific method has been developed to identify the etiologic or immunogenic agent responsible for the production of autoantibodies characteristic of a particular disorder or immune response. The antigen is first isolated, then divided into overlapping short amino acid sequences. The sequences having the greatest reactivity with the autoantibodies are identified and compared with all known amino acids sequences using the available computer data bases. The protein having the maximum number of sequences homologous to the sequences of greatest reactivity with the autoantibodies is the likeliest candidate for the etiological agent. Applying this method, it has been determined that the etiological agent for the production of anti-Ro/SSA autoantibodies characteristic of numerous autoimmune diseases such as SLE appears to be a virus highly homologous to the Indiana strain of the vesicular stomatitis virus. Once the etiologic agent and antigenic sequences are known, it is possible to design assays and reagents for the diagnosis and treatment of patients having either the etiological agent and/or autoantibodies. An animal model has been developed for studying the mechanisms of, and screening compounds for the treatment or prevention of, the expression of these autoantibodies.

AN 97:49507 USPATFULL

TI Assays and treatments of autoimmune diseases

IN Harley, John B., Oklahoma City, OK, United States

PA Oklahoma Medical Research Foundation, Oklahoma City, OK, United States (U.S. corporation)

PI US 5637454 19970610 <--

AI US 1994-335198 19941107 (8)

RLI Continuation of Ser. No. US 1991-648205, filed on 31 Jan 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-472947, filed on 31 Jan 1990, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Saunders, David
LREP Arnall Golden & Gregory
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 1940
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5637454 19970610 <--
DETD . . . demyelinating diseases, multiple sclerosis, subacute cutaneous lupus erythematosus, hypoparathyroidism, Dressler's syndrome, myasthenia gravis, autoimmune thrombocytopenia, idiopathic thrombocytopenic purpura, hemolytic anemia, **pemphigus vulgaris**, pemphigus, dermatitis herpetiformis, alopecia arcata, **pemphigoid**, scleroderma, progressive systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), adult onset diabetes mellitus (Type II. . .

DETD Twelve and one-half percent (12.5%) polyacrylamide **gels** with a 4.5% polyacrylamide stacking **gel** with 0.2% sodium dodecyl sulfate were employed utilizing discontinuous buffer conditions for analysis of peptides by Western immunoblotting. All samples. . . sulfate and 10% 2-mercaptoethanol for 5 min prior to electrophoresis. Digests of purified bovine Ro/SSA were analyzed by 12.5% polyacrylamide **gel** electrophoresis using the method of Mamula, et al., J. Exp. Med. 86:1889-1901 (1986). Protein samples were then electroblotted onto nitrocellulose. . . Mo.) conjugated to alkaline phosphatase was added (at 1:7500 dilution) in 0.1M Tris, 0.1M NaCl, 0.005M MgCl.sub.3 at pH 9.5. **Gels** were then exposed to substrate, 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Promega Corporation, Madison, Wis.), for 2-10 minutes, allowing development of. . .

DETD For sequence analysis, Staphylococcal V-8 protease digests (100 .mu.g/lane) were electrophoresed on 12.5% **gels** and electroblotted onto polyvinylidene difluoride membranes, which have been shown to provide excellent solid phase support for sequencing in automated. . .

DETD . . . 10,000.times.g for 10 minutes. Next, the virus is pelleted through a 50% glycerol cushion at 85,000.times.g for 90 minutes. The **pellet** is then suspended in a 10 mM Tris, 0.1M NaCl, and 0.001M EDTA (TNE) solution, and sonicated at 40 watts. . .

DETD . . . non-specific in that it cannot be directed at the underlying cause. Immunosuppressants which are currently in use include glucocorticoids, methotrexate, **azathioprine**, cyclophosphamide, non-steroidal antiinflammatory agents, antimalarials, and other non-specific therapeutics such as sun screens. Usage and dosage of these drugs is. . . by the disease manifestations. Glucocorticoids, for example, are used in high dosages to treat some neurologic complications of SLE. Both **azathioprine** and cyclophosphamide are used as an attempt to halt or reverse renal damage. Limiting side effects are common for all. . .

L9 ANSWER 34 OF 68 USPATFULL
AB Four triterpenes of Formula 1 (where "---" is either a single or double bond and R is H or acetate) are disclosed which are potent and selective immunosuppressive agents. These compounds have been isolated from Spachea correa root. ##STR1##
AN 97:42907 USPATFULL
TI Triterpenes
IN Goetz, Michael A., Scotch Plains, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5631282 19970520 <--
AI US 1995-476806 19950607 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Ivy, C. Warren; Assistant Examiner: Smith, Lyman H.
LREP Camara, Valerie J., Daniel, Mark R.
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 414
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5631282 19970520 <--
SUMM . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, Graves ophthalmopathy and asthma.
DETD . . . they may also be administered, either alone or together with comprising an antiproliferative agent selected from the group consisting of: **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester, cyclosporin, FK-506 and rapamycin, or other compounds which would be co-administered to. . .

DETD . . . This was first fractionated by preparative thin layer chromatography (TLC) on a 20 cm by 20 cm E. Merck silica **gel** 60F.sub.254 plate of 1 mm thickness using methylene chloride--ethyl acetate 1:1 (v/v) as solvent, then by high performance liquid chromatography. . .
DETD Homogeneity of the preparations was ascertained in several TLC systems, such as E. Merck silica **gel** 60F.sub.254, methylene chloride-ethyl acetate 1:1, Rf 1(a) 0.4, Rf 1(b) 0.3; Whatman KC.sub.18, methanol-water 9:1, Rf 1(a) 0.65, Rf 1(b). . .
DETD Partial purification of the methylene chloride extract was achieved by column chromatography on E. Merck silica **gel** 60 (120 ml), eluting with a step gradient of ethyl acetate in methylene chloride. The step gradient was designed so. . . afforded 100 mg and 20 mg respectively of 1(a) and 1(b) after crystallization from methanol. Later-eluting fractions from the silica **gel** column above were found to contain at least two related compounds based on UV spectra and color reactions on TLC. . .
DETD . . . chloride each time. The pooled methylene chloride extracts are evaporated down and fractionation proceeds by repeated column chromatography on silica **gel**. One employs methylene chloridemethanol 97:3 in a first step; the mixed compounds of Formula 1(a) and 1(b) thus obtained are resolved by chromatographing on fresh silica **gel** eluted with methylene chloride-ethyl acetate 3:1. Volume of elution for the compound of Formula 1(a) ranges from about 2 to. . .

CLM What is claimed is:
8. The pharmaceutical formulation of claim 7, comprising in addition, an
antiproliferative agent selected from the group consisting of: **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester, cyclospofin, FK-506 and rapamycin.

. . . (e) extracting the aqueous solution of (d) with methylene chloride; (f) chromatographing the methylene chloride extract of (e) on silica **gel** using a step gradient of ethyl acetate in methylene chloride for elution wherein the steps comprise the use of 100%. . .

L9 ANSWER 35 OF 68 USPATFULL
AB A method for the treatment of a cutaneous, ocular, or mucosal pathological condition which is associated with an immune response in a human or other mammal, that includes **topical** application of an effective amount of buspirone or a buspirone derivative or its pharmaceutically acceptable salt, optionally in a pharmaceutically-acceptable diluent or carrier for **topical** application.
AN 97:42648 USPATFULL
TI **Topical** application of buspirone for treatment of pathological conditions associated with immune responses
IN Sharpe, Richard J., Cambridge, MA, United States
Arndt, Kenneth A., Newton Centre, MA, United States
Galli, Stephen J., Winchester, MA, United States
PA Beth Israel Deaconess Medical Center, Inc., Boston, MA, United States (U.S. corporation)
PI US 5631017 19970520 <--
AI US 1993-37225 19930326 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Venkat, Jyothsna
LREP Kilpatrick & Cody, L.L.P.
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 741
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI **Topical** application of buspirone for treatment of pathological conditions associated with immune responses
PI US 5631017 19970520 <--
AB . . . ocular, or mucosal pathological condition which is associated with an immune response in a human or other mammal, that includes **topical** application of an effective amount of buspirone or a buspirone derivative or its pharmaceutically acceptable salt, optionally
in a pharmaceutically-acceptable diluent or carrier for **topical** application.
SUMM This invention is in the area of the **topical** treatment of cutaneous, ocular, and mucosal hypersensitivity and hyperproliferative conditions induced by or associated with an immune response, that includes. . .
SUMM . . . Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due
to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, lichen
planus, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions.. . .

SUMM . . . agents with partial utility for treating some of the above conditions include psoralen plus ultraviolet A (PUVA), cyclosporin A, or **azathioprine**, but the risk-to-benefit ratios for these agents is unfavorable for most of the conditions described above.

SUMM It is an object of the present invention to present a method for the **topical** treatment of cutaneous, mucosal and ocular pathology associated with immune responses.

SUMM It is yet another object of the present invention to present a method for the **topical** treatment of cutaneous, mucosal, or ocular hypersensitivity and epithelial hyperproliferation.

SUMM It is yet another object of the invention to present a method for the **topical** treatment of cutaneous, mucosal or ocular scarring.

SUMM . . . mucosal condition in a human or other mammal resulting from pathology associated with an immune response is provided that includes **topical** application of an effective amount of buspirone or a buspirone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.

SUMM . . . exhibits a strong immunosuppressive activity when applied topically. The parent buspirone is used herein as the model of an active **topical** immunosuppressant. Buspirone derivatives are measured against this model, and are considered to be immunosuppressants if they suppress the ear swelling. . .

SUMM . . . suitable carrier in an amount sufficient to effectively immunosuppress the patient at the site of application. Because the application is **topical**, i.e., local, immunosuppression is achieved without producing significant systemic effects, most notably, the significant neuroleptic effect that is associated with. . .

SUMM Buspirone and its active derivatives are administered as general immunosuppressive agents. The compounds may be useful as **topical** agents in treating contact dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions. The novel. . .

DETD Mammals, and specifically humans, suffering from pathological cutaneous, ocular, or mucosal immune responses can be treated by **topical** administration to the patient of an effective amount of the buspirone derivative or its salt, optionally in combination with a. . .

DETD Solutions or suspensions for **topical** application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,. . .

DETD Suitable vehicles or carriers for **topical** application are known, and include lotions, suspensions, **ointments**, **creams**, **gels**, tinctures, sprays, powders, pastes, slow-release transdermal patches, aerosols for asthma, suppositories for application to rectal, vaginal, nasal or oral mucosa,. . .

DETD Thickening agents, emollients, and stabilizers can be used to prepare **topical** compositions. Examples of thickening agents include petrolatum, beeswax, xanthan gum, or polyethylene glycol, humectants such as sorbitol, emollients such as mineral oil, lanolin and its derivatives, or squalene. A number of solutions and **ointments**

are commercially available, especially for ophthalmic and dermatologic applications.

DETD Natural or artificial flavorings or sweeteners can be added to enhance the taste of **topical** preparations applied for local effect to mucosal surfaces. Inert dyes or colors can be added, particularly in the case of. . .

DETD . . . potential irritancy or neuropharmacological effects of the composition. See, in general, Arndt, K. A., P. V. Mendenhall, "The Pharmacology of **Topical** Therapy", Dermatology in General Medicine, 1987; T. B. Fitzpatrick, A. Z. Eisen, K. Wolff, I. M. Freedberg and K. F.. . .

DETD Buspirone and buspirone derivatives are capable of suppressing the immune response in humans and other mammals on **topical** application. As such, the compounds, or therapeutic compositions thereof, may be useful for the treatment of a myriad of immunological. . .

DETD **Topical** Buspirone Treatment For these experiments, both ears of each mouse were challenged for elicitation of contact hypersensitivity by the application. . . ears of control mice were similarly treated, but with vehicle alone. In the case of experiments designed to evaluate the **topical** effect of buspirone on the sensitization phase, only the right ear is challenged (see FIGS. 9 and 10).

DETD Mice were sensitized to oxazolone as described in Example 1. Three days later, slow release indomethacin **pellets** (0.05 mg, 3 week release) were implanted subcutaneously under light ether anesthesia. The dose of indomethacin delivered by these **pellets** has been previously shown to completely block prostaglandin synthesis in mice, by Jun, D. D., et al., J. Invest. Dermatol.. . .

DETD . . . of 50 mM Tris HCl buffer pH 7.7 at 25.degree. C. and centrifuged at 49,000.times. g for 10 min. The **pellet** is resuspended in fresh buffer and incubated at 37.degree. C. for 10 min. After the final centrifugation, the **pellet** is resuspended in 80 volumes of Krebs-HEPES buffer (25 mM HEPES, 118 mM NaCl, 5 mM KCl, 2.5 mM CaCl.sub.2,. . .

DETD . . . and variations of the present invention relating to methods for the treatment of pathology associated with immune responses that includes **topical** administration of an effective amount of buspirone or a buspirone derivative will be obvious to those skilled in the art. . .

CLM What is claimed is:

. . . of a cutaneous, ocular, or mucosal pathology associated with an immune response in a human or other mammal that includes **topical** application of an effective amount of buspirone or its pharmaceutically acceptable salt, other than a quaternary salt, optionally in a pharmaceutically acceptable diluent or carrier for **topical** application.

L9 ANSWER 36 OF 68 USPATFULL

AB The present invention provides compositions comprising a peptide having between about 7 and about 20 amino acid residues, the peptide being capable of binding a CD8 molecule on a cytolytic T lymphocyte (CTL) precursor and inhibiting differentiation of the CTL precursor to a mature CTL. The peptides have amino acid sequences substantially homologous to a sequence in an .alpha.3 domain of a human Class I MHC

molecule. The sequence from the .alpha.3 domain is preferably between residue 220 and residue 235. The peptides typically comprise the sequences DQTQDTE (SEQ. ID No. 1) or EDQTQDTELVETRP (SEQ. ID No. 2).
AN 97:31678 USPATFULL
TI Methods of using CD8 binding domain peptides
IN Clayberger, Carol, Stanford, CA, United States
Krensky, Alan M., Stanford, CA, United States
PA The Board of Regents of the Leland Stanford Junior University, Stanford,
CA, United States (U.S. corporation)
PI US 5620956 19970415 <--
AI US 1994-279501 19940722 (8)
RLI Continuation of Ser. No. US 1991-791925, filed on 8 Nov 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Davenport, Avis M.
LREP Morrison & Foerster LLP
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 6 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 855
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5620956 19970415 <--
DETD A related use for the present invention is in modulating the immune response involved in "graft versus host" disease (**GVHD**). **GVHD** is a potentially fatal disease which occurs when immunologically competent cells are transferred to an allogeneic recipient. If the donor's. . . recipient. Tissues of the skin, gut epithelia and liver are frequent targets and may be destroyed during the
course of **GVHD**. The disease presents an especially severe problem when immune tissue is being transplanted, such as in bone marrow
transplantation; but less severe **GVHD** has also been reported in other cases as well, including heart and liver transplants. Applied in the **GVHD** context, the peptides of the present invention are used to block the binding domain on the CD8 molecules of the. . .
DETD . . . the supernatant. Another method involves concentrating the suspension by ultrafiltration, then resuspending the concentrated liposomes in a replacement medium. Alternatively, **gel** filtration can be used to separate large liposome particles from solute molecules.
DETD The pharmaceutical compositions are intended for parenteral, **topical**, oral or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment. The compositions are suitable for. . .
DETD . . . al., supra), T cell antigens (e.g., OKT4 and OKT3), antithymocyte globulin, as well as chemotherapeutic agents such as cyclosporine, glucocorticoids, **azathioprine**, prednisone and the like may be used in conjunction with the peptides.

L9 ANSWER 37 OF 68 USPATFULL
AB Novel macrolide compounds of the formula ##STR1## and pharmaceutically acceptable salts, esters, amides and prodrugs thereof, wherein X is a substituent selected from among radicals having the subformulae ##STR2##
and other heterocyclic radicals, as well as pharmaceutical compositions and methods of immunomodulatory treatment utilizing the same.
AN 97:22793 USPATFULL

TI Macrocyclic immunomodulators with novel cyclohexyl ring replacements
IN Or, Yat S., Libertyville, IL, United States
Luly, Jay R., Libertyville, IL, United States
PA Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)
PI US 5612350 19970318 <--
AI US 1995-424912 19950419 (8)
RLI Division of Ser. No. US 1994-334454, filed on 8 Nov 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-159406, filed on 30 Nov 1993, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Crowley, Steven R., Steele, Gregory W.
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2032
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5612350 19970318 <--
SUMM . . . is beneficial as well. These other immunosuppressant agents include but are not limited to FK-506, rapamycin, cyclosporin A, mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide.
DETD . . . (3.times.150 mL), brine (300 mL), dried over magnesium sulfate and solvent remove in vacuo. The product was purified by silica **gel** chromatography (1.25 kg) eluting with 12% acetone in hexanes. Yield: 70.3 g; MS (FAB) m/z: M+H=1022.
DETD . . . 0.degree. C. for 1 hour, solids were filtered off and solvent removed in vacuo. The residue was purified by silica **gel** chromatography (15 g) eluting ether. The crude product was further purified by silica **gel** chromatography (100 g) eluting with 10% acetone in hexanes. Yield: 12.3 g; MS (FAB) m/z: M+K=944.
DETD . . . 1 N hydrochloric acid, brine, dried over magnesium sulfate and solvent removed in vacuo. The product was purified by silica **gel** chromatography (50 g) eluting with 10% acetone/hexanes followed by 20% acetone/hexanes. Yield: 7.7 g; MS (FAB)m/z: M+K=942.
DETD . . . washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The crude residue Was purified by silica **gel** (125 g) chromatography eluting with 30% acetone in hexanes. Yield: 6.4 g; MS (FAB) m/z: M+K=844.
DETD . . . repeated and stirring continued for an additional 3 hours. Solvent was removed in vacuo. The solid was purified by silica **gel** chromatography eluting with 70% acetone in hexanes. Yield: 6.4 g; MS (FAB) m/z: M+K=830.
DETD . . . washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The solid residue was purified by silica **gel** chromatography (90 g) eluting with 5% isopropanol in dichloromethane. Yield: 4.65 g; MS (FAB) m/z: M+K=946.
DETD . . . was washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product was purified by silica **gel** chromatography (5 g) eluting with 40% acetone in hexanes. Yield: 0.56 g.
DETD . . . for an additional hour. Solid was removed by filtration and solvent removed in vacuo. The solid was purified by silica **gel** chromatography (12 g) eluting with 35% acetone in hexanes. Yield: 0.24 g; MS (FAB) m/z: M+K=921. m.p. 150.degree.-159.degree. C.
DETD . . . room temperature. After being stirred at room temperature overnight, solvent was removed in vacuo. The product was purified by silica **gel** chromatography (20g) eluting with 30% acetone in hexanes. Yield: 0.46 g; MS (FAB) m/z: M+K=928.

DETD . . . and stirred for an additional hour. Solid was filtered off and solvent removed in vacuo. Product was purified by silica **gel** chromatography (4 g) eluting with 30% acetone in hexanes. Yield: 0.13 g;
MS (FAB) m/z: M+K=814. m.p. 115.degree.-118.degree. C.

DETD . . . over magnesium sulfate and solvent removed in vacuo (safety shield!). The crude acyl azide (19.6 g) was purified by silica **gel** chromatography (250 g) eluting with 20% acetone in hexanes. Yield: 17.4 g.

DETD . . . tetrahydrofuran (30 mL) was refluxed under nitrogen for 3 hours. Solvent was removed in vacuo and product purified by silica **gel** chromatography. Yield: 2 g; MS (FAB) m/z: M+K=941.

DETD . . . minutes. After being stirred at 0.degree. C. for 30 minutes, the reaction mixture is applied on a column of silica **gel** (50 g) in 40% acetone/hexanes and eluted with 40% acetone/hexanes to give the title compound.

DETD . . . with 1N sodium bicarbonate, brine, dried over magnesium sulfate
and solvent removed in vacuo. The product is purified by silica **gel** chromatography.

DETD . . . dry tetrahydrofuran (25 mL) at room temperature for 1 hour. Solvent is removed in vacuo and product purified by silica **gel** chromatography.

DETD . . . was washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product was purified by silica **gel** chromatography (25 g) eluting with 30% acetone/hexanes. Yield: 0.26 g; MS (FAB) m/z: M+K=943.

DETD . . . temperature for 1.5 hour, the solids were filtered off and solvent removed in vacuo. The intermediate was purified by silica **gel** chromatography. Yield: 0.56 g. The intermediate (0.56 g) was dissolved in dry THF (5 mL) under nitrogen at room temperature.. . . THF solution and stirred at room temperature overnight. Solvent was removed in vacuo and the product was purified by silica **gel** chromatography (13 g) eluting with 30% acetone in hexanes. Yield: 0.55 g.

DETD . . . stirred at 70.degree. C. for 1hour. After being cooled down to room temperature, the reaction mixture was poured to silica **gel** (25 g) and eluted with 10% acetone/hexanes followed by 30% acetone/hexanes. Yield: 0.34 g; MS (FAB) m/z: M+K=984.

DETD . . . room temperature for 10 days. Solids are filtered off and solvent removed in vacuo. The product is purified by silica **gel** chromatography.

DETD . . . being stirred at room temperature for 0.5 hour, the solvent was
removed in vacuo and the product purified by silica **gel** chromatography (150 g) eluting with 40% acetone/hexanes. Yield: 1.7 g; MS (FAB) m/z: M+K=1028.

DETD . . . phase washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product was purified by silica **gel** chromatography (50 g) eluting with 20% acetone/hexanes. Yield: 0.953 g; MS (FAB) m/z: M+K=1010.

DETD . . . O.sub.3 for 3 hours. The solids were filtered off and solvent removed in vactto. The product was purified by silica **gel** chromatography (25 g) during with 25 % ethyl acetate/methylene chloride.
Yield: 0.6 19 g.

DETD . . . bicarbonate. The organic phase was dried over magnesium sulfate
and solvent removed in vacuo. The product was purified by silica **gel** chromatography (25 g) eluting with 18% acetone in hexanes.

Yield: 0.35 g; MS (FAB) m/z: M+K=941.

DETD . . . invention are useful when used alone, combination therapy with other immunosuppressants, such as, FK506, rapamycin, cyclosporin A, picibanil, mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide, is also beneficial.

DETD . . . of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise). . .

DETD . . . or formulation auxiliary of any type, which may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, **ointments**, drops or transdermal patch), bucally, or as an oral or nasal spray. The term "parenteral" as used herein refers to. . .

DETD **Topical** administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for **topical** administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized. . .

DETD A further form of **topical** administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as autoimmune diseases, allergic. . . body, aqueous humor, vitreous humor, cornea, iris/cilary, lens, choroid/retina and sclera. The pharmaceutically-acceptable ophthalmic vehicle may, for example, be an **ointment**, vegetable oil or an encapsulating material.

L9 ANSWER 38 OF 68 USPATFULL

AB The present invention relates to intercellular adhesion molecules (ICAM-1) which are involved in the process through which lymphocytes recognize and migrate to sites of inflammation as well as attach to cellular substrates during inflammation. The invention is directed toward such molecules, screening assays for identifying such molecules and antibodies capable of binding such molecules. The invention also includes uses for adhesion molecules and for the antibodies that are capable of binding them.

AN 97:22659 USPATFULL

TI Nucleotide sequence encoding intercellular adhesion molecule-1 and fragments thereof

IN Springer, Timothy A., Newton, MA, United States
Rothlein, Robert, Danbury, CT, United States
Marlin, Steven D., Danbury, CT, United States
Dustin, Michael L., University City, MO, United States

PA Dana Farber Cancer Institute, Boston, MA, United States (U.S. corporation)

PI US 5612216 19970318 <--

AI US 1994-186456 19940125 (8)

RLI Division of Ser. No. US 1990-515478, filed on 27 Apr 1990, now patented, Pat. No. US 5284931 And a continuation-in-part of Ser. No. US 1987-45963, filed on 4 May 1987, now abandoned Ser. No. Ser. No. US 1987-115798, filed on 2 Nov 1987, now abandoned Ser. No. Ser. No. US 1988-155943, filed on 16 Feb 1988, now abandoned Ser. No. Ser. No. US 1988-189815, filed on 3 May 1988, now abandoned Ser. No. Ser. No. US 1988-250446, filed on 28 Sep 1988, now abandoned Ser. No. Ser. No. US 1989-324481, filed on 16 Mar 1989, now abandoned Ser. No. Ser. No. US

1989-373882, filed on 30 Jun 1989, now abandoned And Ser. No. US 1989-456647, filed on 22 Dec 1989, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Cunningham, Thomas M.

LREP Sterne, Kessler, Goldstein & Fox P.L.L.C.

CLMN Number of Claims: 10

ECL Exemplary Claim: 1

DRWN 33 Drawing Figure(s); 25 Drawing Page(s)

LN.CNT 5205

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5612216 19970318 <--

SUMM (b) at least one immunosuppressive agent selected from the group consisting of: dexamethesone, **azathioprine** and cyclosporin A.

DETD . . . such a screen. Thus, for example, the antigen bound by the antibody may be analyzed as by immunoprecipitation and polyacrylamide **gel** electrophoresis. If the bound antigen is a member of the LFA-1 family of molecules then the immunoprecipitated antigen will be. . .

DETD . . . a Teflon Potter Elvejhem homogenizer, and then centrifuged at 1000.times. g for 15 minutes. The supernatant was retained and the **pellet** was re-extracted with 200 ml of 2.5% Tween 40 in Tris-saline. After centrifugation at 1000.times.g for 15 minutes, the supernatants from both extractions were combined and centrifuged at 150,000.times.g for 1 hour to **pellet** the membranes. The membranes were washed by resuspending in 200 ml Tris-saline, centrifuged at 150,000.times.g for 1 hour. The membrane **pellet** was resuspended in 200 ml Tris-saline and was homogenized with a motorized homogenizer and Teflon pestle until the suspension was. . .

DETD . . . be used in structural studies, a column of 10 ml of RR1/1-Sepharose CL-4B (coupled at 2.5 mg of antibody/ml of **gel**), and two 10 ml pre-columns of CNBr-activated, glycine-quenched Sepharose CL-4B, and rat-IgG coupled to Sepharose CL-4B (2 mg/ml) were used.. . .

DETD Approximately 200 .mu.g of purified ICAM-1 was subjected to a second stage purification by preparative SDS-polyacrylamide **gel** electrophoresis. The band representing ICAM-1 was visualized by soaking the **gel** in 1M KCl. The **gel** region which contained ICAM-1 was then excised and electroeluted according to the method of Hunkapiller et al., Meth. Enzymol. 91:227-236. . .

DETD ICAM-1 purified from human spleen migrates in SDS-polyacrylamide **gels** as a broad band of M.sub.r of 72,000 to 91,000. ICAM-1 purified from JY cells also migrates as a broad. . .

DETD . . . to Eco R1 linkers (New England Biolabs), digested with Eco R1 and size selected on a low melting point agarose **gel**. cDNA greater than 500 bp were ligated to .lambda.gt10 which had previously been Eco R1 digested and dephosphorylated (Stratagene) The. . .

DETD . . . the manufacturers recommended quantity of Bam H1 and Eco R1 endonucleases (New England Biolabs). Following electrophoresis through a 0.8% agarose **gel**, the DNAs were transferred to a nylon membrane (Zeta Probe, BioRad). The filter was prehybridized and hybridized following standard procedures. . . 20 .mu.g of total RNA or 6 .mu.g of poly(A).sup.+ RNA. RNA was denatured and electrophoresed through a 1% agarose-formaldehyde **gel** and electrotransferred to Zeta Probe. Filters were prehybridized and hybridized as described previously (Staunton, D. E., at al. Embo J.. . .

DETD . . . diseases were studied for their expression of ICAM-1 and HLA-DR. A proportion of keratinocytes in biopsies of allergic contact

eczema, **pemphigoid**/pemphigus and lichen planus expressed ICAM-1. Lichen planus biopsies showed the most intense staining with a pattern similar to or even. . .

DETD . . . No. of ICAM-1 HLA-DR ICAM-1 &
Diagnosis Cases Only Only HLA-DR

Allergic Contact				
	5	.sup. 3.sup.a		
			0	2
Eczema				
Lichen Planus				
	11	3	0	8
Pemphigoid/				
	2	2	0	0
Pemphigus				
Exanthema	3	2	0	0
Urticaria	4	1	0	1

.sup.a Samples were considered as positive if at. . .

DETD . . . and anti-LFA-1 antibodies. In order to determine whether the combined administration of anti-ICAM-1 and other immunosuppressive agents (such as dexamethasone, **azathioprine**, cyclosporin A or steroids (such as, for example, prednisone, etc.) would also have enhanced effects, MLR assays were performed using. . .

DETD . . . the inhibitory effects of R6-5-D6 are at least additive with the inhibitory effects of suboptimal doses of dexamethasone (Table 19), **Azathioprine** (Table 20) and cyclosporin A (Table 21). This implies that anti-ICAM-1 antibodies can be effective in lowering the necessary doses. . .

DETD TABLE 20

Effect of Anti-ICAM-1 and **Azathioprine** on the Human MLR

Group	Inhibitor (ng/ml)	3HT Incorporation (CPM)	% Inhibition
Media	--	78	--
Stimulators (S)	--	174	--
Responders (R)	--	3,419	--
R .times. S	--	49,570	--
R .times. S	R6-5-D6 (8)	44,374	11
R .times. S	**Azathioprine** (1)	42,710	14
R .times. S	R6-5-D6 (8) + **Azathioprine** (1)	34,246	31

L9 ANSWER 39 OF 68 USPATFULL

AB Immunomodulatory macrocyclic compounds having the formula: ##STR1## and pharmaceutically acceptable salts, esters, amides and prodrugs thereof, as well as pharmaceutical compositions containing such compounds and therapeutic methods of their use.

AN 97:14709 USPATFULL
TI Substituted thiol macrolactam immunomodulators
IN Or, Yat S., Libertyville, IL, United States
Luly, Jay R., Libertyville, IL, United States
PA Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)
PI US 5604234 19970218 <--
AI US 1995-529862 19950918 (8)
RLI Continuation-in-part of Ser. No. US 1993-149416, filed on 9 Nov 1993, now patented, Pat. No. US 5457111 which is a continuation-in-part of Ser. No. US 1993-32958, filed on 17 Mar 1993, now abandoned which is a continuation-in-part of Ser. No. US 1991-755208, filed on 5 Sep 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Crowley, Steven R., Steele, Gregory W.
CLMN Number of Claims: 26
ECL Exemplary Claim: 1,21
DRWN No Drawings
LN.CNT 4174
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5604234 19970218 <--
DETD . . . magnesium sulfate and concentrated in vacuo to give 837 mg of crude product. This material was, purified twice by silica **gel** column chromatography eluting with 0.5% methanol in chloroform to afford the title compound (165 mg). MS (FAB) m/z: M+K=888. IR(KBr). . .
DETD . . . carbonate and brine and dried over magnesium sulfate. After the solvent is removed, the crude product is purified on silica **gel** column chromatography.
DETD . . . anhydrous ether (4.times.50 mL). The combined ether extracts were concentrated in vacuo, and the solid residue was purified by silica **gel** chromatography eluting with 5% acetone in hexanes to provide the title compound (17 g). MS (FAB) m/z: M+H =1022.
DETD . . . residue, and the mixture was dried over magnesium sulfate, filtered and concentrated in vacuo. The product was purified by silica **gel** (20 g) chromatography eluting with 20% (v/v) acetone in hexanes to afford 0.67 g of the title compound. MS (FAB). . .
DETD . . . the total disappearance of starting material is observed. The solvent is evaporated, and the crude product is purified by silica **gel** column chromatography to yield the protected title compound.
DETD . . . brine and then dried over anhydrous magnesium sulfate. Evaporation of the solvent gives crude product which is purified by silica **gel** (25 g) column chromatography eluting with 1.5% methanol in chloroform.
DETD . . . is stirred at room temperature for 5 hours. The solvent is removed, and the crude product is purified by silica **gel** column chromatography to yield the title compound.
DETD . . . washed with 10% KHSO.sub.4. The organic layer was washed with brine, dried over anhydrous Na.sub.2 SO.sub.4, filtered through a silica **gel** plug and concentrated in vacuo to give 34.2 g of 32-trifluoromethanesulfonyl ascomycin in 97% yield.
DETD . . . anhydrous Na.sub.2 SO.sub.4 and concentrated in vacuo to afford 34.03 g of crude product which was purified on a silica **gel** plug to give 25.32 g of the tittle compound in 83% yield. m.p. 93.degree.-96.degree. C. MS (FAB) m/e 846 (M+K).sup.+.. . .

DETD . . . in vacuo to afford 310 mg of crude product, which was dissolved in methylene chloride and filtered through a silica **gel** plug. The partially purified product was eluted with 30:70 acetone-hexane and then further purified by HPLC. on a microsorb column. . .

DETD . . . the solvent was removed under reduced pressure to afford 1.1 g of crude material. Purification by column chromatography on silica **gel** eluting with 15% acetone in hexane afforded the title compound. MS (FAB) m/e 887 (M+K).sup.+. Anal calcd for C.sub.46 H.sub.73. . .

DETD . . . chloroacetone. After .about.2 hours at room temperature, the solvent was removed under reduced pressure. Purification by column chromatography on silica **gel** eluting with 25% acetone in hexane afforded 4 g of title compound. A portion of the product was further purified. . .

DETD . . . at room temperature, the solvents were removed under reduced pressure. The residue obtained was purified by column chromatography on silica **gel** eluting with 1:1 acetone-hexane to give 156 mg of 81A and 390 mg of the isomeric 81B. The 81B fraction. . .

DETD . . . an additional hour at room temperature, the solvents were removed in vacuo. The residue obtained was filtered through a silica **gel** plug eluting with 1:1 acetone-hexane to give 675 mg of partially purified material. This material was further purified by NP-HPLC. . .

DETD . . . removed by filtration and the flitrate concentrated in vacuo. The crude material obtained was passed through a plug of silica **gel** eluting with 40% acetone in hexane to afford 685 mg of partially purified product. This material was further purified by. . .

DETD . . . then concentrated under reduced pressure and dried to constant weight to provide 850 mg of crude product. Purification by silica **gel** column chromatography eluting with 25% acetone in hexane gave 498 mg of the title compound. m.p. 93.degree.-95.degree. C. MS (FAB). . .

DETD . . . C. for .about.1 hour and at room temperature for 2 hours. The crude material was purified by chromatography on silica **gel** eluting with 15% acetone in hexane to afford the title compound. m.p. 116.degree.-118.degree. C. MS (FAB) m/e 930 (M+K).sup.+. Anal. . .

DETD . . . C. for .about.1 hour and at room temperature for 2 hours. The crude material was purified by chromatography on silica **gel** eluting with 15% acetone in hexane to afford the title compound. MS (FAB) m/e 916 (M+K).sup.+. Anal calcd for C47H.sub.75. . .

DETD . . . C. for .about.1 hour and at room temperature for 2 hours. The crude material was purified by chromatography on silica **gel** eluting with 18% acetone in hexane to afford the title compound. MS (FAB) m/e 950 (M+K).sup.+. Anal calcd for C.sub.50. . .

DETD . . . C. for 30 minutes and at room temperature for 2.5 hours. The crude material was purified by chromatography on silica **gel** eluting with 15% acetone in hexane to afford the title compound. MS (FAB) m/e 902 (M+K).sup.+. Anal calcd for C.sub.46. . .

DETD . . . was filtered cold through a bed of Celite and concentrated in vacuo. The residue obtained was filtered through a silica **gel** plug eluting with 30% isopropanol in methylene chloride. The partially purified material was further purified on RP-HPLC. MS (FAB) m/e. . .

DETD . . . with brine, dried over magnesium sulfate and concentrated in vacuo. The residue obtained was filtered through a plug of silica **gel** eluting with 30% acetone in hexane to give partially purified material. This material was further purified by NP-HPLC on a. . .

DETD . . . the solvent was removed under reduced pressure to afford 630 mg of crude product. Purification by column chromatography on silica **gel** eluting with 25% acetone in hexane gave 400 mg of the title compound. MS (FAB) m/e 917 (M+K).sup.+. .sup.13 C. . .

DETD . . . then concentrated under reduced pressure. The residue obtained was dried to a constant weight (900 mg) and purified by silica **gel** column chromatography eluting with 25% acetone in hexane to give 310 mg of the title compound. MS (FAB) m/e 917. . .

DETD . . . brine. The organic layer was dried over MgSO.sub.4 and concentrated to give 2.2 g of crude product. Purification on silica **gel** column chromatography eluting with 30% acetone in hexane afforded 860 mg of the title compound. MS (FAB) m/e 1067 (M+K).sup.+.. . .

DETD . . . hours. The solvent was removed in vacuo, and the residue was dried to constant weight (1.13 g). Purification by silica **gel** column chromatography eluting with 25% acetone in hexane afforded 420 mg of the title compound. MS (FAB) m/e 1047 (M+K).sup.+.. . .

DETD . . . of p-toluenesulfonic acid monohydrate. The reaction was stirred for 6 hours. The reaction mixture was then filtered through a silica **gel** plug eluting with 40% acetone in hexane. The eluant was concentrated to give 629 mg of crude product. Purification by silica **gel** column chromatography eluting with 32% acetone in hexane gave 213 mg of the title compound. MS (FAB) m/e 931 (M+K).sup.+.. . .

DETD . . . reaction-was concentrated under reduced pressure, and the residue was dried to give 750 mg of crude product. Purification by silica **gel** column chromatography eluting with 25% acetone in hexane provided 500 mg of the title compound. MS (FAB) m/e 918 (M+K).sup.+.. . .

DETD . . . dried to constant weight to give 1 g of crude product. After filtration of the crude material through a silica **gel** plug by elution with 50% acetone in hexane, the filtrate was concentrated under reduced pressure. Purification by NP-HPLC using a. . . .

DETD . . . reduced pressure and dried to give 419 mg of crude product. After filtration of the crude material through a silica **gel** plug by elution with 60% acetone in hexane, the filtrate was concentrated under reduced pressure to provide 250 mg of. . .

DETD . . . concentrated under reduced pressure, and the residue was dried to give 2.1 g of crude product. After purification by silica **gel** column chromatography eluting with 20% acetone in hexane 1.3 g of the title compound was obtained.

DETD . . . g of a crude product mixture containing the isomeric title compounds and the sulfonyl compound. Initial purification was by silica **gel** column chromatography eluting with 50% acetone in hexane. Final purification was performed on a Rainin Microsorb NP-HPLC column eluting with. . .

DETD . . . under reduced pressure and the residue dried to constant weight to give 2.6 g of crude product. Purification by silica **gel** column chromatography eluting with 20% acetone in hexane gave 1.39 g of pure title compound. MS (FAB) m/e 922 (M+K).sup.+.. . .

DETD . . . to constant weight to give 975 mg of crude product as a mixture of sulfoxide isomers. After purification by silica **gel** column chromatography eluting with 35% acetone in hexane, 236 mg of isomer 133A and 600 mg of the other isomer. . .

DETD . . . immunosuppressants is beneficial as well. These other agents

include but are not limited to FK-506, rapamycin, cyclosporin A, mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide.

DETD . . . invention are useful when used alone, combination therapy with other immunosuppressants, such as, FK506, rapamycin, cyclosporin A, picibanil, mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide, is also beneficial.

DETD . . . of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise). . .

DETD . . . formulation auxiliary of any type. The compositions may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, **ointments**, drops or transdermal patch), bucally, or as an oral or nasal spray. The term "parenteral" as used herein refers to. . .

DETD **Topical** administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for **topical** administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized.

In non-pressurized. . .

DETD A further form of **topical** administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as automimmue diseases, allergic. . . body, aqueous humor, vitreous humor, cornea, iris/cilary, lens, choroid/retina and sclera. The pharmaceutically-acceptable ophthalmic vehicle may, for example, be an **ointment**, vegetable oil or an encapsulating material.

L9 ANSWER 40 OF 68 USPATFULL

AB O-Aryl, O-alkyl, O-alkenyl and O-alkynyl-macrolides of the general structural Formula I: ##STR1## have been prepared from suitable precursors by alkylation and/or arylation at C-3" and/or C-4" of the cyclohexyl ring. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases and/or the prevention of rejection of foreign organ transplants and/or related afflictions, diseases and illnesses.

AN 96:94689 USPATFULL

TI O-Aryl,O-alkyl,O-alkenyl and O-alkynylmacrolides having immunosuppressive activity

IN Goulet, Mark, Westfield, NJ, United States
Organ, Helen M., Fanwood, NJ, United States
Parsons, William H., Edison, NJ, United States
Sinclair, Peter J., Highland Park, NJ, United States
Wong, Frederick, Glen Ridge, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5565560 19961015 <--

AI US 1993-132072 19931004 (8)

RLI Continuation-in-part of Ser. No. US 1992-875036, filed on 1 May 1992, now patented, Pat. No. US 5250678, issued on 5 Oct 1993 which is a continuation-in-part of Ser. No. US 1991-809998, filed on 18 Dec 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-699407, filed on 13 May 1991, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Bond, Robert T.
LREP Yang, Mollie M., Rose, David L.
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 7386
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5565560 19961015 <--
SUMM . . . of foreign organ transplants, (e.g. bone marrow, kidney, liver, heart, skin, small-bowel, and pancreatic islet-cell transplants, including xeno transplants), the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as: psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus or Alopecia areata), male pattern alopecia, alopecia senilis, reversible obstructive. . .
SUMM . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyperproliferative skin diseases and of cutaneous manifestations of immunologically-mediated illness. A Fisons World patent application (PCT. . .
SUMM . . . onset diabetes, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .
SUMM . . . the supression of in vitro immune systems (J. Antibiotics 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).
SUMM . . . 3,644,364 and 4,098,791. Upjohn United States Patents (U.S. Pat. Nos. 4,139,619 and 4,596,812) discloses the use of minoxidil in the **topical** treatment of human baldness. Similarly, an Upjohn United States Patent (U.S. Pat. No. 5,026,691) discloses the use of minoxidil and an antiinflammatory agent for the treatment of patterned male and female alopecia. Japanese patent Kokai 61-260010 states that **topical** minoxidil formulations containing other specified agents may be prepared. An Upjohn WIPO patent application (PCT Publication No. WO 92/09259) discloses. . . University of Miami WIPO patent application (PCT Publication No. WO 92/12703) discloser a method of stimulating hair growth comprising the **topical** application of a phospholipid.
SUMM . . . chloroform, benzene, toluene and the like. The triarylbismuth(V) reagent can be used without purification or can be purified by silica **gel** chromatography. Triarylbismuthines may be prepared by the reaction of an appropriate aryl Grignard reagent with bismuth trichloride in an inert. . .
SUMM . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises,

seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne Alopecia areata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .

SUMM . . . parenteral applications. The active ingredient may be compounded, for example, with the usual non- toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .

SUMM . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of **azathioprine** (AZA), brequinar sodium, deoxyspergualin (DSG), mizaribine, mycophenolic acid morpholino ester (RS-61443), cyclosporin and rapamycin.

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 3:4 EtOAc/hexanes to afford 46 mg of 17-ethyl-1,14-dihydroxy 12-[2'-(4"-phenyloxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR and mass. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The products were separated and purified by flash column chromatography on silica **gel** [eluted with 4:1 hexanes/acetone followed by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone] to yield 94 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-phenyloxy-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 110 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-phenyloxy-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13, 19, 21, 27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 3:1 hexanes/EtOAc) to afford 39 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-fluorophenyloxy)-3"-methoxycyclohexyl)-1'-methyl-vinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR and mass spectral. . .

DETD . . . Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was separated and purified two times by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to give 40 mg 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-chlorophenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/EtOAc) to give 47 mg 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-methylphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in

vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 31 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4"'-methylphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-methylphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR. . .

DETD . . . dried over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (2:1 hexanes/acetone) to give 66 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-phenoxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis were. . .

DETD . . . over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified 3.times. by preparative TLC on silica **gel** (3:2 hexanes/acetone) to afford 35 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-phenoxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4"'-phenoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C. . .

DETD . . . Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified 2 times by preparative TLC on silica **gel** (3:1 hexanes/acetone) to give 38 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-1-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR analysis was consistent with the desired structure).

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 3:1 hexanes/acetone) to yield 49 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(naphth-1-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-aza-tricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 39 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-1-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-1,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone: (.sup.1 H NMR. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (3:1 hexanes/acetone) to afford 32 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yloxy)-3"-

methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis were. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 3:1 hexanes/acetone) to give 63 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(napth-2-yloxy)-4"-hydroxy-cyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 49 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by two preparative thin layer chromatographys on silica **gel** (first chromatography eluted with 2:1 hexanes/acetone, isolated band at R.sub.f =0.26 second chromatography eluted with 3.5% methanol/CH.sub.2 Cl.sub.2, isolated band. . .

DETD . . . The mixture was filtered and concentrated in vacuo. The triarylbismuthine is isolated and purified by flash column chromatography on silica **gel**.

DETD . . . dissolved in several milliliters of 4:1 hexanes/acetone plus small amount of CH.sub.2 Cl.sub.2. The solution was passed through a silica **gel** plug and eluted with 4:1 hexanes/acetone. The filtrate was concentrated in vacuo. The residue was dissolved in 4:1 hexanes/acetone plus small amount of CH.sub.2 Cl.sub.2 and passed through a second silica **gel** plug and eluted with 4:1 hexanes/acetone. The filtrate was concentrated in vacuo leaving 52 mg yellow residue that was used. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative thin layer chromatography on silica **gel** (eluted with 2:1 hexanes/acetone) to give 7.1 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6"'-methoxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.35) and 9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(6"'-methoxy-naphth-2-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f. . .

DETD . . . (4 mL) was added bis(trifluoroacetoxy)iodobenzene (162 mg, 0.377 mmol). The mixture was stirred 5 minutes, then passed through a silica **gel** plug and eluted with EtOAc. The eluant was concentrated in vacuo. The residue was dissolved in CH.sub.2 Cl.sub.2 (4 mL). . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (2:1 hexanes/acetone) to afford 26.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-methoxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.35). (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . (3 mL) was added bis(trifluoroacetoxy)iodobenzene (162 mg, 0.377 mmol). The mixture was stirred 5 minutes, then passed through a

silica **gel** plug and eluted with EtOAc. The eluant was concentrated in vacuo. The residue was dissolved in CH.sub.2 Cl.sub.2 (4 mL). . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by radial chromatography on silica **gel** (2 mm plate eluted with 3:1 hexanes/acetone) and then by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 78.4 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3"'-methoxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.40). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 47 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6"'-tert-butyldimethylsilyloxynaphth-2-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.56). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 44.2 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6"'-hydroxynaphth-2-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.23). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 81 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-tertbuytl-dimethylsilyloxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27'-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ne-2,3,10,16-tetraone (R.sub.f =0.49). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 52 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-hydroxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.25). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 15.5 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-methylthiophenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.47). (.sup.1 H NMR and mass spectral were. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 23.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(2"'-methylphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-

tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9
]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.46). (.sup.1 H NMR and
mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and
concentrated in vacuo. The products were isolated by radial
chromatography on silica **gel** (eluted with 3:1 hexanes/ethyl
acetate) to afford 70.9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3"'-

methylphenyloxy)-3"-methoxycyclohexyl)-1'-methyl-vinyl]-23,25-dimethoxy-
13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9
]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral
analysis were. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and
concentrated in vacuo. The products were isolated by radial
chromatography on silica **gel** (eluted with 3.5%
methanol/CH.sub.2 Cl.sub.2) and then purified by preparative TLC on
silica **gel** (eluted with 3:1 hexanes/acetone) to afford 24.3 mg
of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3"',4"'-dimethylphenyloxy)-3"-
methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9
]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral
analysis were consistent. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered,
and
concentrated in vacuo. The products were separated by preparative TLC
on
silica **gel** (2:1 hexanes/acetone). Each compound was repurified
2.times. by preparative TLC on silica **gel** (3:1 hexanes/acetone
then 3.5% MeOH/CH.sub.2 Cl.sub.2) affording 23.4 mg of
17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-methoxyphenyloxy)-3"-
hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo-22.3.1.0.sup.4,9
]octacos-18-ene-2,3,10,16-tetraone and 28.4 mg of 17-ethyl-1,14-
dihydroxy-12-[2'-(3"-(4"'-methoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-
methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-
azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.
(.sup.1
H. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered,
and
concentrated in vacuo. The products were separated by preparative TLC
on
silica **gel** (2:1 hexanes/acetone). Each compound was repurified
2.times. by preparative TLC on silica **gel** (2:1 hexanes/acetone
then 3.5% MeOH/CH.sub.2 Cl.sub.2) affording 27 mg of
17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3"'-methoxyphenyloxy)-3"-
hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-
tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9
]octacos-18-ene-2,3,10,16-tetraone and 35 mg of
17-ethyl-1,14-dihydroxy-

12-[2'-(3"-(3"'-methoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-
23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-
azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.
(.sup.1
H. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered,
and
concentrated in vacuo. The products were separated by preparative TLC
on

silica **gel** (2:1 hexanes/acetone) affording 41.9 mg. of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-tert-butyldimethylsilyloxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42.5 mg. of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4"'-tert-butyldimethylsilyloxyphenyloxy)-4"-hydroxycyclo-hexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) affording 25.7 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4"'-hydroxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were consistent with. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) affording 23.9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4"'-hydroxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis are consistent with. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated by preparative TLC on silica **gel** (2:1 hexanes/acetone) affording 39.8 mg. of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6"'-tert-butyldimethylsilyloxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 41.6 mg. of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(6"'-tert-butyldimethylsilyloxynaphth-2-yl-oxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted 2.times. with 2:1 hexanes/acetone) affording 17 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6"'-hydroxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted 2.times. with 2:1 hexanes/acetone) affording 20.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(6"'-hydroxynaphth-2-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1

H NMR and mass spectral analysis were consistent. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (3:2 EtOAc/hexanes) and a second preparative TLC (eluted 2.times. with 3:1 hexanes/acetone) affording 24.7 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(ethoxycarbomethoxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H. . .

DETD . . . dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 2:1 hexane/acetone to give 12 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(phenanthr-9-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR was consistent with the desired. . .

DETD . . . with anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 2:1 Hexane/Acetone) to give 37 mg of

17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3"',4"'-methylenedioxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . combined, dried with anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (eluted with 2:1 Hexane/Acetone) to give 14 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(2"',3"'-dihydrobenzofuran-5-yl)-3"-methoxycyclohexyl)-1'-methylvinyl-]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone characterized by (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 3:1 Hexane/Acetone) to give 234 mg of 17-allyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . were combined, dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (eluted with 4% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 18 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(1"',4"'-benzodioxane-6-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and mass. . .

DETD . . . combined organic washes were dried with magnesium sulphate and concentrated. The crude residue was purified by column chromatography on silica **gel** eluting with70% hexane: 30% ethyl acetate to give the title compounds A (93mg) and B (102mg) each as white solids.

DETD . . . Cl.sub.2. The extracts were combined, dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. Purified by preparative TLC on silica **gel** (eluted with 7% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 22 mg of 17-ethyl-1,2,14-trihydroxy-12-[2'-(4"-(naphth-

2-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-3,10,16-trione (.sup.1 H NMR and mass. . .

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (156 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compound (17 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compound (10 mg).

DETD . . . at room temperature. After 1.5 hours, the mixture was filtered over Celite, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) to give the title compound (19.5 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (21 mg 4"-ether; 17 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (15 mg 4"-ether; 16 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (11 mg 4"-ether; 13 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (14 mg 4"-ether; 12 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (24 mg 4"-ether; 21 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (34 mg 4"-ether; 24 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compound (17 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the

title compound (12 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (11 mg 4"-ether; 13 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (45 mg).

DETD . . . room temperature. After 30 minutes, the mixture was filtered over diatomacous earth, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) to give the title compound (5.5 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (13 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (9 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (8 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (16 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (10 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (17 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (20 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (33 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (34 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (19 mg).

DETD . . . at room temperature. After 45 minutes, the mixture was filtered over Celite, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) to give the title compound (7.5 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (6.8 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . at room temperature. After 25 minutes, the mixture was filtered over Celite, concentrated and purified by flash chromatography on silica **gel** (ethyl acetate: hexane (1:3)+1% methanol) to give the title compound (4.5 mg).

DETD . . . brine and the organic phase dried over magnesium sulfate. Removal of the solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate: hexane (1:3)+1% methanol) gave the title compound (2.91 g). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (1.51 g). (1 H NMR was consistent with the desired structure).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ether:hexane (2:3)) gave the title compound (800 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride:hexane:methanol (10:2:1)) to give the title compound (300 mg) (.sup.1 H NMR was consistent. . .

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (151 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . extracted with ethyl acetate (3.times.5 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound (3.5 mg).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) gave the title compound (2 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:3)+1% methanol) gave the title compound (320 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride: hexane:methanol (10:2:1)) to give the title compound (232 mg).

(.sup.1 H NMR was. . .

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (112 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . extracted with ethyl acetate (3.times.5 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound (2.1 mg).

DETD . . . extracted with ethyl acetate (3.times.15 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1+1% methanol) to give the title compound (80.2 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:3)+1% methanol) gave the title compound (24 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (4 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (92 mg).

DETD . . . combined organics are washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** gives the title compound.

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification oof the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (190 mg).

DETD . . . ethyl acetate. The combined organics were dried over magnesium sulfate, concentrated in vacuo and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1) to give the title compound (50 mg).

DETD . . . The organics were dried by passage through a magnesium sulfate plug and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol then (1:1+1% methanol) to give the title compound (13 mg).

DETD . . . mg) and the reaction stirred at room temperature. After 30 minutes the mixture was filtered through a small diatomaceous earth/silica **gel** plug and the filtrate concentrated in vacuo. Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (10 mg).

DETD . . . and brine. The combined organics were dried over magnesium sulfate and concentrated in vacuo. Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the desired product (145 mg).

DETD . . . organics were dried by passage through a magnesium sulfate plug, concentrated in vacuo and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (43 mg).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash

chromatography on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compound (6 mg).

DETD . . . and the organic portion washed with brine, dried over magnesium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (3:2) to give the title compound (8.4 g)

DETD . . . sodium bicarbonate, brine, and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (10% acetone in hexane) gave the title compounds (3" ether: 1.81 g, 4" ether: 1.20 g).

DETD . . . and the organic portion washed with brine, dried over magnesium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1+1% methanol) to give the title compound (316 mg).

DETD . . . (5.5 mg), and the mixture stirred at room temperature. After 15 minutes, the mixture was filtered through a small silica **gel** column, washed with ethyl acetate, and the concentrated organics purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (282 mg).

DETD . . . washed with water. The organic portion was dried over sodium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (4:1)+1% methanol+0.5% acetic acid) to give the title compound (43 mg).

DETD . . . colored persisted. The mixture was then warmed to room temperature, concentrated in vacuo, and purified by flash chromatography on silica **gel** (acetone:hexane (1:2)) to give the title compound (5.5 mg).

DETD . . . at room temperature for 12 hours. At this time the mixture was concentrated and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (43 mg).

DETD . . . ml), and the combined organic portions washed with brine, dried over magenesium sulfate and purified by flash chromatography on silica **gel** (2% methanol in methylene chloride followed by 2% methanol in methylene chloride+0.5% acetic acid) to give the title compound (255. . .

DETD . . . sodium bicarbonate. The organic portion was dried over magnesium sulfate, concentrated in vacuo, and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then (2:1)+1% methanol) to give the title compound (14 mg).

DETD . . . extracted with ethyl acetate, and the organics dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (5 mg).

DETD . . . with ethyl acetate, and the organic portion dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) gave the title compound (74 mg).

DETD . . . with ethyl acetate, and the organic portion dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol, in methylene chloride) gave the title compound (10 mg).

DETD . . . (2 ml) dropwise. The reaction mixture was stirred for 15

minutes after the addition and then filtered through a silica **gel** pad washing with ethyl acetate. The filtrate was concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired product (188 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 50% hexane:50% ethyl acetate to give the title compound (102 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired product (216 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 70% hexane:30% ethyl acetate to give the title compound (11 mg).

DETD . . . acetate. The organic extracts were dried (MgSO.sub.4) and concentrated and the crude material was purified by column chromatography on silica **gel** eluting with 65% hexane:35% ethyl acetate to give the desired product (22 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 50% hexane:50% ethyl acetate to give the title compound (15 mg).

DETD . . . stirred at room temperature for 48 hours. The reaction was then diluted with ethyl acetate and filtered through a silica **gel** pad. The filtrate was concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired compound (12.6 mg).

DETD . . . brine and extacted with ethyl acetate. The organic extracts were dried (MgSO.sub.4), concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired compound (27 mg).

DETD . . . washed with saturated sodium chloride solution, and the organic portion dried over magnesium sulfate. Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) followed by silica **gel** preparative tic (acetone:hexane 2:8) gave the title compound (2.8 mg).

DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2 / minutes at 4.degree. C. Cold medium was added and cells were again. . .

L9 ANSWER 41 OF 68 USPATFULL

AB Immunomodulatory macrocyclic compounds having the formula: ##STR1## and pharmaceutically acceptable salts, esters, amides and prodrugs thereof,

where R.sup.8 and R.sup.9 are selected such that one of R.sup.8 and R.sup.9 is hydrogen and the other is --S(O).sub.s --heterocyclic,

as well as pharmaceutical compositions containing such compounds and therapeutic methods of their use.

AN 96:89850 USPATFULL

TI Thio-heterocyclic macrolactam immunomodulators

IN Or, Yat S., Libertyville, IL, United States

Luly, Jay R., Libertyville, IL, United States
PA Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)
PI US 5561137 19961001 <--
AI US 1994-212621 19940314 (8)
RLI Continuation-in-part of Ser. No. US 1993-32958, filed on 17 Mar 1993, now abandoned which is a continuation-in-part of Ser. No. US 1991-755208, filed on 5 Sep 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Danckers, Andreas M., Crowley, Steven R.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1122
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5561137 19961001 <--
SUMM . . . to be beneficial as well. These other agents include but are not limited to FK-506, rapamycin, cyclosporin A, mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide.
SUMM . . . would be useful when used alone, combination therapy with other
immunosuppressants, such as, FK506, rapamycin, cyclosporin A, picibanil,
mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide, would also be expected to be beneficial.
SUMM . . . of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidennolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, ache and Alopecia areata; various eye diseases (autoimmune and otherwise). . .
SUMM . . . formulation auxiliary of any type. The compositions may be administered orally, rectally, parenterally, intracisternally, intravagmally, intraperitoneally, topically (as by powders, **ointments**, drops or transdermal patch), bucally, or as an oral or nasal spray. The term "parenteral" as used herein refers to. . . .
SUMM **Topical** administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for **topical** administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In
non-pressurized. . .
SUMM A further form of **topical** administration is to the eye, as for the treatment of immunemediated conditions of the eye such as autoimmune
diseases, allergic. . . body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera. The pharmaceutically-acceptable ophthalmic vehicle may, for example, be an **ointment**, vegetable oil or an encapsulating material.
DETD . . . organic phase was washed once with ice-cold brine and dried over magnesium sulfate. The filtrate was poured on a silica **gel** column (50 g) prepacked in ether and eluted with ether. Solvent was removed in vacuo to give the title compound. . .
DETD . . . washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The crude product was purified by silica **gel** chromatography (90 g) eluting with 27% acetone/hexanes.

Yield: 2.0 g, m.p. =96.degree.-98.degree. C.; MS (FAB) m/e M+K=830.

DETD . . . was added and the reaction mixture was stirred at room temperature for 24 hours. The product was purified by silica **gel** chromatography (70 g) eluting with 10% acetone-hexanes. Yield: 0.5 g; m.p. =106.degree.-110.degree. C.; MS (FAB) m/e M+NH.sub.4 =907.

DETD . . . in dichloromethane (10 mL) at 0.degree. C. After being stirred at room temperature overnight, the reaction was purified by silica **gel** chromatography (250 g) eluting with 40% acetone-hexanes. Yield: 1.4 g; m.p. =92.degree.-96.degree. C.; MS (FAB) m/e M+H=888.

DETD . . . cesium carbonate (0.16 g) in dichloromethane (1.5 mL) and stirred at room temperature overnight. The product was purified by silica **gel** chromatography (3 g) eluting with 70% acetone in hexanes. Yield: 0.093 g; MS (FAB) m/e M+K=942.

L9 ANSWER 42 OF 68 USPATFULL

AB Aryl, alkyl, alkenyl and alkynyl macrolides of the general structural Formula I: ##STR1## have been prepared from suitable precursors by oxidation and alkylation at C-4" of the cyclohexyl ring. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases and/or the prevention of rejection of foreign organ transplants and/or related afflictions, diseases and illnesses.

AN 96:77884 USPATFULL

TI Aryl, alkyl, alkenyl and alkynylmacrolides having immunosuppressive activity

IN Rupprecht, Kathleen M., Cranford, NJ, United States
Baker, Robert K., Cranford, NJ, United States
Ok, Hyun O., Edison, NJ, United States
Parsons, William H., Edison, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5550233 19960827 <--

AI US 1994-263298 19940621 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Ford, John M.; Assistant Examiner: Sripada, Pavanaram K.

LREP Thies, J. Eric, Rose, David L.

CLMN Number of Claims: 13

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 6334

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5550233 19960827 <--

SUMM . . . of foreign organ transplants, (e.g. bone marrow, kidney, liver, heart, skin, small-bowel, and pancreatic islet-cell transplants, including xeno transplants), the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as: psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus or Alopecia arecata), male pattern alopecia, alopecia senilis, reversible obstructive. . .

SUMM . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyperproliferative skin diseases and of cutaneous manifestations of

immunologically-mediated illness. A Fisons World patent application (PCT. . .

SUMM . . . onset diabetes, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .

SUMM . . . the supression of in vitro immune systems (J. Antibiotics 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).

DETD . . . toluene and the like. The triaryl- or triheteroarylbismuth(V) reagent may be used without purification or may be purified by silica **gel** chromatography. Triaryl- or triheteroarylbismuthines may be prepared by the reaction of an appropriate aryl or heteroaryl grignard reagent with bismuth. . .

DETD . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne Alopecia arcata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .

DETD . . . parenteral applications. The active ingredient may be compounded, for example, with the usual non- toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .

DETD . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of **azathioprine** (AZA), brequinar sodium, deoxyspergualin (DSG), mizaribine, mycophenolic acid morpholino ester (RS-61443), cyclosporin and rapamycin.

DETD . . . with water and saturated sodium chloride solution, dried with anhydrous magnesium sulfate and concentrated. The residue was chromatographed on silica **gel** (66% ethyl acetate: 33% hexane: 1% methanol) to give 350 mg of product. This material was dissolved in 10 ml. . .

DETD . . . under a nitrogen atmosphere. The solvent was removed under reduced pressure and the dark residue was purified by chromatography (silica **gel**, 7% i-propanol/CH.sub.2 Cl.sub.2) to give 17-ethyl-1-hydroxy-12-[2'-(4",3"-dihydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,-27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-14,18-diene-2,3,10,16-tetraone (180mg) as a white solid. This material was dissolved in ethanol (20 ml). . . introduced via balloon for 30 min. and the mixture was filtered through celite. Removal of solvent followed by chromatography (silica **gel**) gave 172 mg of the title compound. Mass, .sup.1 H and 13C NMR data were consistent with the title structure.

DETD . . . layer was washed (water, sat'd NaHCO.sub.3, sat'd NaCl) and dried (anhydrous MgSO.sub.4). Removal of solvent followed by chromatography on silica **gel** (70% hexane/ethyl acetate) gave 150 mg of product. MASS: (FAB) 1110 (M+ + Li).

DETD . . . sodium bicarbonate and extracted with ethyl acetate three times. Normal workup and removal of solvent followed by purification on silica **gel** column (80% ethyl acetate/hexane) gave 560 mg of

the title compound as a white solid. MASS: (FAB) 954 (M+ +. . .

DETD . . . quenched with saturated sodium bicarbonate, then extracted with

ethyl acetate. Removal of solvent in vacuo followed by chromatography on

silica **gel** (80% ethyl acetate/hexane) gave 300 mg of product (Mass, 1 H and 13C NMR data consistent with the title compound.

DETD . . . with brine and the organic phase dried over magnesium sulfate. Removal of solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (235 mg). (1H NMR consistent with the desired structure).

DETD . . . acetate, washed with brine and dried over magnesium sulfate. The solution was concentrated and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (89 mg).(1H NMR consistent with the desired structure).

DETD . . . was warmed to room temperature. Extraction from ethyl acetate, drying over magnesium sulfate and purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% MeOH) gave the title compound (22 mg). (.sup.1 H NMR consistent with the desired structure).

DETD . . . the organic phase dried by passage through a magnesium sulfate column. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound. MASS:(FAB) 816 (M+Na). Partial .sup.13 C NMR .delta.: 211.5 (C-16); 196.1 (2). . .

DETD . . . the organic phase dried by passage through a magnesium sulfate column. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) gave the title compound.

DETD . . . KHCO.sub.3, and brine. The two layers were combined, dried over

MgSO.sub.4, and concentrated. The oily residue was purified by silica **gel** chromatography with 10% ether:hexane to afford 0.313 g (93%) of the title compound. MS (FAB) 1086 (M+Li).

DETD . . . partitioned between H.sub.2 O and diethyl ether. The organic fraction was dried over MgSO.sub.4, filtered and concentrated in vacuo. Silica **gel** chromatography with 15% Ethyl acetate:hexane gave 210 mg of title compound. MASS: (FAB) 1068 (M.sup.+ + Li).

DETD . . . dried over MgSO.sub.4, filtered and the filtrate was concentrated in vacuo. The oily residue was purified by flash chromatography (silica **gel**, 6 cm.times.20 cm) using ethyl acetate-hexane to afford 3.28 g (63%) of the title compound as a colorless foam; NMR. . .

DETD . . . of 5 mL of trimethylethoxy-silane and the solution was concentrated under vacuum. The residue was purified by flash chromatography (silica **gel**, 1.5 cm.times.10 cm) using 20% acetoneohexane and the product lyophilized from benzene to afford 0.152 g (85%) of the title. . .

DETD . . . saturated KHCO.sub.3 solution and brine, dried over MgSO.sub.4,

filtered and concentrated. The oily residue was purified by flash chromatography (silica **gel**, 6 cm.times.20 cm) using ethyl acetate-hexane to afford 2.58 g (49%) of the title compound as a colorless foam; MS. . .

DETD . . . of 5 mL of trimethylethoxy-silane and the solution was concentrated under vacuum. The residue was purified by flash chromatography (silica **gel**, 1.5 cm.times.10 cm) using acetone-hexane and the product lyophilized from benzene to afford 0.148 g (82%) of the title compound. . .

DETD . . . was left at room temperature overnight. The next day, the solution was applied to a 15 mL pad of silica **gel** packed with hexane. The pad was washed with 50 mL of hexane, until all of the tin residues had been. . . the product was eluted with 1:3:6 acetonitrile:methyl-tert-butylether:hexanes and concentrated. The residue was purified by flash chromatography (1 cm.times.10 cm, silica **gel**) with 20% ethyl acetate:hexane to afford 0.102 g (45%) of the title compound as a colorless foam. .sup.1 H NMR. . .

DETD . . . brine, then the combined extract was dried over MgSO.sub.4, filtered and concentrated. The oily residue was purified by HPLC (silica **gel**, Waters RCM) using 1:3:6 acetonitrile: methyl-tert-butylether:hexanes to afford 2.36 g (46%) of the title compound as a colorless foam; NMR. . .

DETD . . . brine, then the combined extract was dried over MgSO.sub.4, filtered and concentrated. The oily residue was purified by HPLC (silica **gel**, Waters RCM) using 1:3:6 acetonitrile:methyl-tert-butylether:hexane to afford 3.15 g (61%) of the title compound as a colorless foam; NMR (CDCl.sub.3). . .

DETD . . . brine and then dried over MgSO.sub.4. The solution was concentrated to an oil that was purified by chromatography on silica **gel** using 20% acetone-hexane to afford 0.220 g (67%) of the title compound as a colorless foam. MASS 1188 (M+Li).

DETD . . . with 5 mL of ethoxytrimethylsilane and the solution was concentrated. The oily residue was purified by HPLC (Waters RCM silica **gel**, 25.times.100 mm) using hexane-methyl t-butyl ether-acetonitrile (6:3:1) to afford 45 mg of the faster diastereomer as a white solid. MASS:. . .

DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2 minutes at 4.degree. C. Cold medium was added and cells were again. . .

L9 ANSWER 43 OF 68 USPATFULL

AB Aryl and heteroaryl macrolides of the general structural Formula I: ##STR1## have been prepared from suitable precursors by olefination at C-27. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases, the prevention of rejection of foreign organ transplants and/or related afflictions, diseases, and illnesses.

AN 96:72978 USPATFULL

TI Aryl and heteroaryl macrolides having immunosuppressive activity

IN Baker, Robert K., Cranford, NJ, United States
Kieczykowski, Gerard R., Westfield, NJ, United States
Ok, Hyun O., Edison, NJ, United States
Parsons, William H., Edison, NJ, United States
Rupprecht, Kathleen M., Cranford, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5545734 19960813 <--

AI US 1994-328225 19941025 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Ford, John M.; Assistant Examiner: Sripada, Pavanaram K.

LREP Thies, J. Eric, Rose, David L.

CLMN Number of Claims: 9

ECL Exemplary Claim: 1

DRWN No Drawings
LN.CNT 2639
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5545734 19960813 <--
SUMM . . . of foreign organ transplants, (e.g. bone marrow, kidney, liver,
heart, skin, small-bowel, and pancreatic islet-cell transplants, including xeno transplants), the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as: psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus or Alopecia areata), male pattern alopecia, alopecia senilis, reversible obstructive. . .

SUMM . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyperproliferative skin diseases and of cutaneous manifestations of immunologically-mediated illness. A Fisons World patent application (PCT. . .

SUMM . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .

SUMM . . . the supression of in vitro immune systems (J. Antibiotics 1987,
40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).

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SUMM . . . parenteral applications. The active ingredient may be compounded, for example, with the usual non- toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .

SUMM . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of **azathioprine** (AZA), brequinar sodium, deoxyspergualin (DSG), mizaribine, mycophenolic acid morpholino ester (RS-61443), cyclosporin and rapamycin.

DETD . . . remove residual salts. The ether was concentrated to afford a yellow solid that was purified by flash chromatography on silica **gel** (2cm.times.25 cm column) using 60% ether-hexane to afford 0.631 g (44%) of the title compound as a white solid. Futher. . .

DETD . . . and dried over MgSO.sub.4. The filtrate was concentrated to a yellow solid that was purified by flash chromatography on silica **gel** (2.5 cm.times.25 cm column) using 80% ether-hexane to afford 0.270 g (16%) of the title compound as a white solid.. . .

DETD . . . and dried over MgSO.sub.4. The filtrate was concentrated to a

yellow solid that was purified by flash chromatography on silica **gel** (5 cm.times.25 cm column) using 60% ether-hexane to afford 2.62 g (32%) of the title compound as a white solid.. . .

DETD . . . was stirred at room temperature for 3 h. The solution was poured onto a 5 cm.times.25 cm column of silica **gel** packed in 10% acetone-hexane and the column was washed with two column volumes of 10% acetone-hexane to remove residual thios.. . .

DETD . . . NaHSO.sub.3, then brine, KHCO.sub.3, and brine. The solution was dried over MgSO.sub.4, concentrated and purified by flash chromatography on silica **gel** (2.5 cm.times.20 cm) using 60% ether-hexane and lyophillized from benzene to afford 0.72 g (84%) of the title compound as. . .

DETD . . . 250 m) using two elutions of 30% ethyl acetate-hexane to afford 0.018 g (23%) of the title compound as a **cream**-colored solid. Mass Spectrum (FAB, Li spike) m/e 799 (M+Li).

DETD . . . was washed with KHCO.sub.3, and brine, dried over MgSO.sub.4, and concentrated. The residue was purified by flash chromatography on silica **gel** (6 cm.times.30 cm) using 10% ether hexane to afford 11.50 g (79%) of the title compound as a colorless oil.. . .

DETD . . . to complete precipitation, filtered. The filtrate was concentrated to dryness and the residue was filtered through a pad of silica **gel** using hexane as eluant. The solution was concentrated to afford 10.42 g (87%) of the title compound as a colorless. . .

DETD . . . C. After 8 h, the solution was diluted with 2 mL of dichloromethane and filtered through a pad of silica **gel** using dichloromethane as eluate. The filtrate was concentrated and the residue was purified by flash chromatography (2 cm.times.20 cm column). . .

DETD . . . C. After 24 h, the solution was diluted with 2 mL of dichloromethane and filtered through a pad of silica **gel** using dichloromethane as eluate. The filtrate was concentrated and the residue was purified by flash chromatography (2 cm.times.20 cm column). . .

DETD . . . C. After 8 h, the solution was diluted with 2 mL of dichloromethane and filtered through a pad of silica **gel** using dichloromethane as eluate. The filtrate was concentrated and the residue was purified by flash chromatography (2 cm.times.20 cm column). . .

DETD . . . water and saturated sodium chloride solution, dried with anhydrous magnesium sulfate and concentrate. The is residue was chromatographed on silica **gel** (66% ethyl acetate: 33% hexane: 1% methanol) to give 350 mg of product. This material was dissolved in 10 ml. . .

DETD . . . under a nitrogen atmosphere. The solvent was removed under reduced pressure and the dark residue was purified by chromatography (silica **gel**, 7% i-propanol/CH.sub.2 Cl.sub.2)to give 17-ethyl-1-hydroxy-12-[2'-(4"-hydroxy-3"-isopropyloxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-14,18-diene-2,3,10,16-tetraone (180 mg) as a white solid. This material was dissolved in ethanol (20 ml). . . introduced via balloon for 30 min. and the mixture was filtered through celite. Removal of solvent followed by chromatography (silica **gel**) gave 172 mg of the title compound. Mass Spectrum, 1H and 13C NMR data were consistant with the title structure.

DETD . . . layer was washed (water, sat'd NaHCO.sub.3, sat'd NaCl) and dried (anhydrous MgSO.sub.4). Removal of solvent followed by chromatography on silica **gel** (70% hexane/ethyl acetate) gave

150 mg of product. Mass Spectrum (FAB): 1110 (M+Li).
DETD . . . sodium bicarbonate and extracted with ethyl acetate three times. Normal work-up and removal of solvent followed by purification on
silica **gel** column (80% ethyl acetate/hexane) gave 560 mg of the product as a white solid. Mass Spectrum (FAB): 954 (M+Li).
DETD . . . quenched with saturated sodium bicarbonate, then extracted with
ethyl acetate. Removal of solvent in vacuo followed by chromatography on
silica **gel** (80% ethyl acetate/hexane) gave 300 mg of product (Mass, .sup.1 H and .sup.13 C NMR data consistent with the title. . .
DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2 minutes at 4.degree. C. Cold medium was added and cells were again. .
.

L9 ANSWER 44 OF 68 USPATFULL
AB O-Aryl, O-alkyl, O-alkenyl and O-alkynyl-macrolides of the general structural Formula I: ##STR1## have been prepared from suitable precursors by alkylation and/or arylation at C-3" and/or C-4" of the cyclohexyl ring. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases and/or the prevention of rejection of foreign organ transplants
and/or related afflictions, diseases and illnesses.
AN 96:58222 USPATFULL
TI O-aryl,O-alkyl, and O-alkenyl-macrolides having immunosuppressive activity
IN Goulet, Mark, Westfield, NJ, United States
Parsons, William H., Edison, NJ, United States
Organ, Helen M., Fanwood, NJ, United States
Sinclair, Peter J., Highland Park, NJ, United States
Wong, Frederick, Glen Ridge, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States
PA Merck Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5532248 19960702 <--
AI US 1995-440180 19950512 (8)
RLI Division of Ser. No. US 1993-132072, filed on 4 Oct 1993 which is a continuation-in-part of Ser. No. US 1992-875036, filed on 1 May 1992, now patented, Pat. No. US 5250678 which is a continuation-in-part of Ser. No. US 1991-809998, filed on 18 Dec 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-699407, filed on 13 May 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Yang, Mollie M., Rose, David L.
CLMN Number of Claims: 3
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 8905
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5532248 19960702 <--
SUMM . . . of foreign organ transplants, (e.g. bone marrow, kidney, liver,
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SUMM . . . onset diabetes, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .

SUMM . . . the supression of in vitro immune systems (J. Antibiotics 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).

SUMM . . . 3,644,364 and 4,098,791. Upjohn United States Patents (U.S. Pat. Nos. 4,139,619 and 4,596,812) discloses the use of minoxidil in the **topical** treatment of human baldness. Similarly, an Upjohn United States Patent (U.S. Pat. No. 5,026,691) discloses the use of minoxidil and an antiinflammatory agent for the treatment of patterned male and female alopecia. Japanese patent Kokai 61-260010 states that **topical** minoxidil formulations containing other specified agents may be prepared. An Upjohn WIPO patent application (PCT Publication No. WO 92/09259) discloses. . . University of Miami WIPO patent application (PCT Publication No. WO 92/12703) discloser a method of stimulating hair growth comprising the **topical** application of a phospholipid.

SUMM . . . chloroform, benzene, toluene and the like. The triarylbismuth(V) reagent can be used without purification or can be purified by silica **gel** chromatography. Triarylbismuthines may be prepared by the reaction of an appropriate aryl Grignard reagent with bismuth trichloride in an inert. . .

SUMM . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne Alopecia areata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .

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brequinar sodium, deoxyspergualin (DSG), mizaribine, mycophenolic acid morpholino ester (RS-61443), cyclosporin and rapamycin.

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 3:4 EtOAc/hexanes to afford 46 mg of 17-ethyl-1,14-dihydroxy 12-[2'-(4"-phenyloxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28 -dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR and. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The products were separated and purified by flash column chromatography on silica **gel** [eluted with 4:1 hexanes/acetone followed by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone] to yield 94 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-phenyloxy-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9] octacos-18-ene-2,3,10,16-tetraone and 110 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-phenyloxy-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 3:1 hexanes/EtOAc) to afford 39 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-fluorophenyloxy)-3"-methoxycyclohexyl)-1'-methyl-vinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR and mass spectral. . .

DETD . . . Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was separated and purified two times by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to give 40 mg 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-chlorophenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/EtOAc) to give 47 mg 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methylphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 31 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-methylphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methylphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR. . .

DETD . . . dried over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (2:1 hexanes/acetone) to give 66 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-phenoxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16tetraone. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral analysis were. . .

DETD . . . over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified 3.times. by preparative TLC on silica **gel** (3:2 hexanes/acetone) to afford 35 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-phenoxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-phenoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR, .sup.13 C. . .

DETD . . . Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified 2 times by preparative TLC on silica **gel** (3:1 hexanes/acetone) to give 38 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-1-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR analysis was consistent with the desired structure).

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 3:1 hexanes/acetone) to yield 49 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(naphth-1-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-aza-tricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 39 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-1-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-1,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (3:1 hexanes/acetone) to afford 32 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 NMR, .sup.13 C NMR, and mass spectral analysis were. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated and purified by preparative TLC on silica **gel** (eluted with 3:1 hexanes/acetone) to give 63 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(napth-2-yloxy)-4"-hydroxy-

cyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 49 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 NMR was. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by two preparative thin layer chromatographys on silica **gel** (first chromatography eluted with 2:1 hexanes/acetone, isolated band at R.sub.f =0.26 second chromatography eluted with 3.5% methanol/CH.sub.2 Cl.sub.2, isolated band. . .

DETD . . . The mixture was filtered and concentrated in vacuo. The triarylbismuthine is isolated and purified by flash column chromatography on silica **gel**.

DETD . . . dissolved in several milliliters of 4:1 hexanes/acetone plus small amount of CH.sub.2 Cl.sub.2. The solution was passed through a silica **gel** plug and eluted with 4:1 hexanes/acetone. The filtrate was concentrated in vacuo. The residue was dissolved in 4:1 hexanes/acetone plus small amount of CH.sub.2 Cl.sub.2 and passed through a second silica **gel** plug and eluted with 4:1 hexanes/acetone. The filtrate was concentrated in vacuo leaving 52 mg yellow residue that was used. . .

DETD . . . over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by preparative thin layer chromatography on silica **gel** (eluted with 2:1 hexanes/acetone) to give 7.1 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-methoxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.35) and 9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(6'"-methoxy-naphth-2-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f. . .

DETD . . . (4 mL) was added bis(trifluoroacetoxy)iodobenzene (162 mg, 0.377 mmol). The mixture was stirred 5 minutes, then passed through a silica **gel** plug and eluted with EtOAc. The eluant was concentrated in vacuo. The residue was dissolved in CH.sub.2 Cl.sub.2 (4 mL). . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (2:1 hexanes/acetone) to afford 26.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methoxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.35). (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . (3 mL) was added bis(trifluoroacetoxy)iodobenzene (162 mg, 0.377 mmol). The mixture was stirred 5 minutes, then passed through a silica **gel** plug and eluted with EtOAc. The eluant was concentrated in vacuo. The residue was dissolved in CH.sub.2 Cl.sub.2 (4 mL). . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by radial chromatography on silica **gel** (2 mm plate eluted with 3:1 hexanes/acetone) and then by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 78.4 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'"-methoxyphenyloxy)-3"-

methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.40). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 47 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-tert-butyldimethylsilyloxynaphth-2-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.56). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 44.2 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-hydroxynaphth-2-yloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.23). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 81 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-tertbuytl-dimethylsilyloxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ne-2,3,10,16-tetraone (R.sub.f =0.49). (.sup.1 H NMR and mass spectral. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 52 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-hydroxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub. f =0.25). (.sup.1 H NMR and mass spectral. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 15.5 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methylthiophenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.47). (.sup.1 H NMR and mass spectral were. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) to afford 23.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(2'"-methylphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (R.sub.f =0.46). (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by radial chromatography on silica **gel** (eluted with 3:1 hexanes/ethyl acetate) to afford 70.9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'"-

methylphenyloxy)-3"-methoxycyclohexyl)-1'-methyl-vinyl]-23,25-dimethoxy-

13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by radial chromatography on silica **gel** (eluted with 3.5% methanol/CH.sub.2 Cl.sub.2) and then purified by preparative TLC on silica **gel** (eluted with 3:1 hexanes/acetone) to afford 24.3 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'",4'"-dimethylphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25 -dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated by preparative TLC on silica **gel** (2:1 hexanes/acetone). Each compound was repurified 2.times. by preparative TLC on silica **gel** (3:1 hexanes/acetone then 3.5% MeOH/CH.sub.2 Cl.sub.2) affording 23.4 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-methoxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 28.4 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-methoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated by preparative TLC on silica **gel** (2:1 hexanes/acetone). Each compound was repurified 2.times. by preparative TLC on silica **gel** (2:1 hexanes/acetone then 3.5% MeOH/CH.sub.2 Cl.sub.2) affording 27 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'"-methoxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 35 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(3'"-methoxyphenyloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25 -dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated by preparative TLC on silica **gel** (2:1 hexanes/acetone) affording 41.9 mg. of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-tert-butyldimethylsilyloxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 42.5 mg. of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-tert-butyldimethylsilyloxyphenyloxy)-4"-hydroxycyclo-hexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1

H NMR and mass spectral. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) affording 25.7 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(4'"-hydroxyphenyloxy)-4"-hydroxycyclohexyl)-1 '-methylvinyl]23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted with 2:1 hexanes/acetone) affording 23.9 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(4'"-hydroxyphenyloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis are consistent with. . .

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The products were separated by preparative TLC on silica **gel** (2:1 hexanes/acetone) affording 39.8 mg. of

17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-tert-butyldimethylsilyloxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone and 41.6 mg. of 17-ethyl-1,14-

dihydroxy-12-[2'-(3"-(6'"-tert-butyldimethylsilyloxynaphth-2-yl-oxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted 2.times. with 2:1 hexanes/acetone) affording 17 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(6'"-hydroxynaphth-2-yloxy)-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The product was isolated by preparative TLC on silica **gel** (eluted 2.times. with 2:1 hexanes/acetone) affording 20.8 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(3"-(6'"-hydroxynaphth-2-yloxy)-4"-hydroxycyclohexyl)-1'-methylvinyl]23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1 H NMR and mass spectral analysis were. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4. The mixture was filtered and concentrated in vacuo. The products were isolated by preparative TLC on silica **gel** (3:2 EtOAc/hexanes) and a second preparative TLC (eluted 2.times. with 3:1 hexanes/acetone) affording 24.7 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(ethoxycarbomethoxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. (.sup.1. . .

DETD . . . dried with Na.sub.2 SO.sub.4, filtered and concentrated in

vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 2:1 hexane/acetone to give 12 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4 "-(phenanthr-9-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR was consistent with the desired. . .

DETD . . . with anhydrous Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 2:1 Hexane/Acetone) to give 37 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(3'",4'"-methylenedioxyphenyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and mass spectral analysis were. . .

DETD . . . combined, dried with anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (eluted with 2:1 Hexane/Acetone) to give 14 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(2'",3'"-dihydrobenzofuran-5-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone characterized by (.sup.1 H NMR and mass spectral analysis. . .

DETD . . . dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (eluted with 3:1 Hexane/Acetone) to give 234 mg of 17-allyl-1,14-dihydroxy-12-[2'-(4"-(naphth-2-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and mass spectral analysis were consistent. . .

DETD . . . were combined, dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (eluted with 4% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 18 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(1'",4'"-benzodioxane-6-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (.sup.1 H NMR and. . .

DETD . . . combined organic washes were dried with magnesium sulphate and concentrated. The crude residue was purified by column chromatography on silica **gel** eluting with 70% hexane:30% ethyl acetate to give the title compounds A (93 mg) and B (102 mg) each as. . .

DETD . . . Cl.sub.2. The extracts were combined, dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. Purified by preparative TLC on silica **gel** (eluted with 7% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 22 mg of 17-ethyl-1,2,14-trihydroxy-12-[2'-(4"-(naphth-2-yl)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-3,10,16-trione (.sup.1 H NMR and mass. . .

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (156 mg).

DETD . . . combined organics were washed with brine and dried over

magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (17 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (10 mg).

DETD . . . at room temperature. After 1.5 hours, the mixture was filtered over Celite, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (19.5 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (21 mg 4"-ether; 17 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (15 mg 4"-ether; 16 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (11 mg 4"-ether; 13 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (14 mg 4"-ether; 12 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (24 mg 4"-ether; 21 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (34 mg 4"-ether; 24 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (17 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (12 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (11 mg 4"-ether; 13 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on

silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (45 mg).

DETD . . . room temperature. After 30 minutes, the mixture was filtered over diatomacous earth, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (5.5 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (13 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (9 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (8 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (16 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (10 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (17 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (20 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (33 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (34 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (19 mg).

DETD . . . at room temperature. After 45 minutes, the mixture was filtered over Celite, concentrated and purified by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (7.5 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (6.8 mg). (1H NMR was consistent with the desired structure).

DETD . . . at room temperature. After 25 minutes, the mixture was filtered over Celite, concentrated and purified by flash chromatography on silica

gel (ethyl acetate:hexane (1:3)+1% methanol) to give the title compound (4.5mg).

DETD . . . brine and the organic phase dried over magnesium sulfate. Removal of the solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (2.91 g). (.sup.1 NMR was consistent with the desired structure).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (1.51 g). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ether: hexane (2:3)) gave the title compound (800 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride:hexane:methanol (10:2:1)) to give the title compound (300 mg)(.sup.1 H NMR was consistent with. . .

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (151 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . extracted with ethyl acetate (3.times.5 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound (3.5 mg).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) gave the title compound (2 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (320 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride:hexane:methanol (10:2:1)) to give the title compound (232 mg). (1H NMR was consistent with. . .

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (112 mg). (.sup.1 NMR was consistent with the desired structure).

DETD . . . extracted with ethyl acetate (3.times.5 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title

compound (2.1 mg).

DETD . . . was extracted with ethyl acetate (3.times.15ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1+1% methanol) to give the title compound (80.2 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (24 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (4 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (92 mg).

DETD . . . combined organics are washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** gives the title compound.

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (190 mg). .sup.1 H NMR spectrum was consistent with the desired structure.

DETD . . . ethyl acetate. The combined organics were dried over magnesium sulfate, concentrated in vacuo and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1) to give the title compound (50 mg).

DETD . . . The organics were dried by passage through a magnesium sulfate plug and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol then (1:1+1% methanol) to give the title compound (13 mg). (.sup.1 H NMR consistent with the. . .

DETD . . . mg) and the reaction stirred at room temperature. After 30 minutes the mixture was filtered through a small diatomaceous earth/silica **gel** plug and the filtrate concentrated in vacuo. Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (10 mg). (.sup.1 H NMR consistent with the desired structure)

DETD . . . and brine. The combined organics were dried over magnesium sulfate and concentrated in vacuo. Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the desired product (145 mg). (.sup.1 H NMR consistent with the desired structure)

DETD . . . organics were dried by passage through a magnesium sulfate plug, concentrated in vacuo and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (43 mg).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (6 mg).

DETD . . . and the organic portion washed with brine, dried over magnesium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (3:2) to give the title compound (8.4 g) .sup.1 H NMR consistent with the desired structure.

DETD . . . sodium bicarbonate, brine, and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (10% acetone in hexane) gave the title compounds (3" ether: 1.81 g, 4" ether: 1.20 g). .sup.1 H NMR consistent. . .

DETD . . . and the organic portion washed with brine, dried over magnesium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1+1% methanol) to give the title compound (316 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . (5.5 mg), and the mixture stirred at room temperature. After 15 minutes, the mixture was filtered through a small silica **gel** column, washed with ethyl acetate, and the concentrated organics purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (282 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . washed with water. The organic portion was dried over sodium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (4:1)+1% methanol+0.5% acetic acid) to give the title compound (43 mg). .sup.1 H NMR consistent with the desired. . .

DETD . . . colored persisted. The mixture was then warmed to room temperature, concentrated in vacuo, and purified by flash chromatography on silica **gel** (acetone:hexane (1:2)) to give the title compound (5.5 mg).

DETD . . . at room temperature for 12 hours. At this time the mixture was concentrated and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1: 1)+1% methanol) to give the title compound (43 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . ml), and the combined organic portions washed with brine, dried over magnesium sulfate and purified by flash chromatography on silica **gel** (2% methanol in methylene chloride followed by 2% methanol in methylene chloride+0.5% acetic acid) to give the title compound (255. . .

DETD . . . sodium bicarbonate. The organic portion was dried over magnesium sulfate, concentrated in vacuo, and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then (2:1)+1% methanol) to give the title compound (14 mg). .sup.1 H NMR consistent with the. . .

DETD . . . extracted with ethyl acetate, and the organics dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (5 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . with ethyl acetate, and the organic portion dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) gave the title compound (74 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . with ethyl acetate, and the organic portion dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol, in methylene chloride) gave the title compound (10 mg). . .sup.1. . .

DETD . . . (2 ml) dropwise. The reaction mixture was stirred for 15

minutes after the addition and then filtered through a silica **gel** pad washing with ethyl acetate. The filtrate was concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired product (188 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 50% hexane:50% ethyl acetate to give the title compound (102 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired product (216 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 70% hexane:30% ethyl acetate to give the title compound (11 mg).

DETD . . . acetate. The organic extracts were dried (MgSO.sub.4) and concentrated and the crude material was purified by column chromatography on silica **gel** eluting with 65% hexane:35% ethyl acetate to give the desired product (22 mg).

DETD . . . The organic phase was dried with magnesium sulphate and concentrated. The crude material was purified by column chromatography on silica **gel** eluting with 50% hexane:50% ethyl acetate to give the title compound (15 mg).

DETD . . . stirred at room temperature for 48 hours. The reaction was then diluted with ethyl acetate and filtered through a silica **gel** pad. The filtrate was concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired compound (12.6 mg).

DETD . . . brine and extacted with ethyl acetate. The organic extracts were dried (MgSO.sub.4), concentrated and purified by column chromatography on silica **gel** eluting with 60% hexane:40% ethyl acetate to give the desired compound (27 mg).

DETD Purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) followed by silica **gel** preparative tlc (acetone:hexane 2:8) gave the title compound (2.8 mg).

DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2 minutes at 4.degree. C. Cold medium was added and cells were again.

L9 ANSWER 45 OF 68 USPATFULL

AB Novel macrolide compounds of the formula ##STR1## and pharmaceutically acceptable salts, esters, amides and prodrugs thereof, processes for the preparation of the compounds of the invention, intermediates useful in these processes, a pharmaceutical composition, and a method of treating immunomodulatory disorders are disclosed.

AN 96:53422 USPATFULL

TI Macrolide immunomodulators

IN Or, Yat S., Libertyville, IL, United States
Luly, Jay R., Libertyville, IL, United States
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PI US 5527907 19960618 <--

AI US 1994-327391 19941026 (8)

RLI Continuation-in-part of Ser. No. US 1993-155064, filed on 19 Nov 1993, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Steele, Gregory W., Crowley, Steven R.
CLMN Number of Claims: 36
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 5893
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5527907 19960618 <--
SUMM . . . is beneficial as well. These other immunosuppressant agents include but are not limited to FK-506, rapamycin, cyclosporin A, mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide.
SUMM . . . OH and R.sup.9 is hydrogen with fluorosulfonyl anhydride or trifluoromethylsulfonyl anhydride, followed by reaction of the resulting sulfonate with silica **gel** or an appropriate base to produce the enol ether, followed by hydrolysis of the enol ether; or
SUMM . . . of formula I where R.sup.8 is --OSO.sub.2 F or --OSO.sub.2 CF.sub.3 and R.sup.9 is hydrogen, in the presence of silica **gel** or appropriate mild acid under conditions suitable for the production of the desired product and hydrolysis of the enol ether.
SUMM A suitable reagent for the dehydration of an activated alcohol is silica **gel** or triethylamine. The reaction may be carried out in a solvent which does not adversely affect the reaction (e.g. diethyl. . .
SUMM In process (mm), a suitable acid for the rearrangement of the activated alcohol is silica **gel**. The reaction may be carried out in a solvent which does not adversely affect the reaction (e.g. diethyl ether, dichloromethane,. . .
DETD . . . 0.1N hydrochloric acid. The organic phase was washed once with saturated brine, dried over magnesium sulfate and filtered through silica **gel** (2 g) eluting with ether. The solvent was removed in vacuo, and the product was stored in the freezer.
DETD . . . was washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product was purified by silica **gel** chromatography (20 g) eluting with 20% acetone/hexanes to afford 0.72 g of the title compound. MS (FAB) m/z: M+K=1117.
DETD . . . mixture was allowed to warm to room temperature and stirred for 2 hours. The reaction mixture was purified by silica **gel** chromatography (70 g) eluting with 25% acetone/hexanes to give 343.2 mg of the title compound. m.p. 115.degree.-199.degree. C. MS (FAB). . .
DETD . . . is washed once with brine, dried over magnesium sulfate, and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 30% acetone/hexanes.
DETD . . . 20 mL of water and 20 mL of saturated NaCl solution, dried over magnesium sulfate and passed through a silica **gel** plug eluting with cold ether. The solvent was removed in vacuo, and the residue was dissolved in 10 mL of. . . mL of saturated NaCl solution, dried over MgSO.sub.4 and concentrated in vacuo. The residue obtained was chromatogrpahed on a silica **gel** (15 g) column eluting with isopropanol in dichlormethane to give 27 1 mg of the title compound.

m.p. -90.degree.-93.degree. C.. . .

DETD . . . 0.1N hydrochloric acid. The organic phase was washed once with saturated brine, dried over magnesium sulfate and filtered through silica **gel** (2 g) eluting with ether. The solvent was removed in vacuo, and the product was stored in the freezer.

DETD . . . The organic phase was washed with saturated NaCl solution, dried over MgSO.sub.4 and passed through a short column of silica **gel** (10 g). The partially purified compound was further purified by HPLC (Rainin Microsorb silica **gel**) eluting with 75% acetone in hexane to afford the title compound. m.p. 105.degree.-109.degree. C. MS (FAB) m/z: M+K=1039. Selected CMR. . .

DETD . . . is added and stirred for another 0.5 hour. The solids are filtered off and the product is purified by silica **gel** chromatography.

DETD Silica **gel** (25 g) was added to a solution of the compound resulting from Example 13 (prepared from 0.53 g of rapamycin). . . then removed in vacuo, and the resulting powder was refrigerated for 8 days at 8.degree. C. The product on silica **gel** was eluted with acetone and the solvent removed in vacuo. The crude product was purified by HPLC (Rainin Microsorb silica **gel**) eluting with 30% acetone/hexanes. MS (FAB) m/z: M+K=920.

DETD Silica **gel** (25 g) was added to a solution of the compound resulting from Example 13 (prepared from 0.53 g of rapamycin). . . then removed in vacuo, and the resulting powder was refrigerated for 8 days at 8.degree. C. The product on silica **gel** was eluted with acetone and solvent removed in vacuo. The crude product was purified by HPLC (Rainin Microsorb silica **gel**) eluting with 30% acetone/hexanes. MS (FAB) m/z: M+K=934.

DETD The title compound was isolated from the reaction mixture on silica **gel** of Example 36. MS (FAB) m/z: M+K=934.

DETD . . . once with saturated sodium chloride solution, dried over MgSO.sub.4 and concentrated in vacuo. The residue was purified on a silica **gel** column eluting with 1:1 acetone-hexane to give 380 mg of partially purified material which was further purified by HPLC eluting. . .

DETD . . . under a nitrogen atmosphere and then partitioned between ether and 0.1N HCl. The organic phase was passed through a silica **gel** plug eluting with Et.sub.2 O. This activated intermediate was dissolved in methylene chloride (8 mL), cooled to -78.degree. C., and. . . Et.sub.2 O and 0.1N HCl. The organic phase was concentrated in vacuo, and the residue obtained purified on a silica **gel** column eluting with 4% isopropanol in methylene chloride to give 159 mg of the title compound. m.p. 111.degree.-116.degree. C. MS. . .

DETD . . . continuing 30 minutes after complete addition. The solvent is removed in vacuo and the residue purified by HPLC on silica **gel** . Fractions containing desired product are pooled, and concentrated, to constant weight under high vacuum to give the desired product.

DETD . . . continuing 30 minutes after complete addition. The solvent is removed in vacuo and the residue purified by HPLC on silica **gel** . Fractions containing desired product are pooled, and concentrated, to constant weight under high vacuum to give the desired product.

DETD . . . C. for an additional 24 hours. The solvent is removed in vacuo and the residue purified by chromatography on silica **gel** to provide the title compound.

DETD . . . of piperidine. After complete consumption of starting material, as evidenced by TLC, the material is purified by chromatography on silica **gel** to provide the title compound.

DETD . . . (2 g) was added and stirring was continued for 30 minutes. The

crude mixture was then passed through a silica **gel** column. This partially purified material was rechromatographed on silica **gel** eluting with 35% acetone in hexane to obtain the title compound (380 mg, 40%) which was recrystallized from ether. m.p.. . .

DETD . . . between Et.sub.2 O and water. The organic phase was dried over magnesium sulfate, concentrated in vacuo and purified by silica **gel** chromatography to afford the title compound. MS (FAB) m/z: M+K=951.

DETD . . . once with brine, dried over magnesium sulfate and the solvent removed in vacuo. The crude product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.

DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.

DETD . . . washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The crude product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.

DETD . . . mL) at 0.degree. C. and refrigerated overnight. Pyridine is removed in vacuo, and the crude mixture is purified by silica **gel** chromatography eluting with 65% acetone in hexanes.

DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . is washed once with brine, dried over magnesium surfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.

DETD . . . washed once with brine, dried over magnesium sulfate and the solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.

DETD . . . washed once with brine, dried over magnesium sulfate and the solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 50% acetone in hexanes.

DETD . . . and water. The organic phase was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by silica **gel** chromatography to give the title compound (189 mg). m.p. 105.degree.-111.degree. C. MS (FAB) m/z: M+K =968.

DETD . . . stirring at room temperature for 16 hours, the solvent is removed in vacuo, and the product is purified by silica **gel** chromatogrphy eluting with 5 % isopropanol in dichloromethane.

DETD . . . stirring at room temperature for 5 hours, the solvent is removed in vacuo, and the product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . 0.5 mL of methanol. The reaction mixture was stirred at room temperature for 36 hours and then chromatographed on silica **gel** eluting with 50% acetone in hexanes to afford 0.277 g of the title compound. m.p. 126.degree.-131.degree. C. MS (FAB) m/z:. . .

DETD . . . g) in dichloromethane-tetrahydrofuran (1:1, 4 mL). The reaction mixture was stirred at room temperature overnight and then chromatographed on silica **gel** eluting with 50% acetone in hexanes to afford 0.45 g of the title compound. m.p. 101.degree.-106.degree. C. MS (FAB) m/z:. . .

DETD . . . dry tetrahydrofuran at room temperature. After stirring at room temperature for 36 hours, the reaction mixture is chromatographed on silica **gel** eluting with 50% acetone in hexanes to afford the title compound.

DETD . . . mL of methanol. The reaction mixture was stirred at room temperature under nitrogen overnight and then poured onto a silica **gel** column and eluted with 35% acetone in hexanes to give

partially purified material. This material was rechromatographed on silica **gel** eluting with 25% acetone in hexanes to afford 462 mg. This material was rechromatographed on silica **gel** eluting with 1:1 ethyl acetate-hexane to afford 108 mg of pure title compound. m.p. 102.degree.-106.degree. C. MS (FAB) m/z: M+K. . .

DETD . . . and water. The organic phase was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by silica **gel** column chromatography eluting with 25% acetone in hexanes to afford partially purified compound which was rechromatographed on silica **gel** eluting with 2% isopropanol in methylene chloride to give pure title compound (270.7 mg). m.p. 94.degree.-98.degree. C. MS (FAB) m/z:. . .

DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . was washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product was purified by silica **gel** chromatography eluting with 40% acetone in hexanes to afford 0.41 g of the title compound. MS (FAB) m/z: M+K =994.

DETD . . . is washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . g) and DDQ (2 equivalent) is stirred in wet dichloromethane at room temperature overnight. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . Example 58 (1 g) in chloroform is stirred at 50.degree.-60.degree. C. for 4 hours. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . minutes. The reaction was then warmed to ambient temperature and stirred for 5 days. The mixture was adsorbed onto silica **gel** by dilution of the mixture with CH.sub.2 Cl.sub.2 (5 mL) followed by addition of silica **gel** (70-230 mesh, 60 A, 5 mL) and solvent evaporation. The adsorbed silica bed was placed on a fresh pad of. .

DETD . . . of Example 99 is treated with dichlorodicyanobenzoquinone in warm benzene. The mixture is concentrated and purified by chromatography on silica **gel** to provide pure title compound.

DETD . . . (257 mg, 1.88 mmol) is added, and stirring is continued overnight. The reaction mixture is purified by chromatography on silica **gel** to provide the title compound.

DETD . . . SO.sub.4), filtered, and the solvent removed in vacuo to give crude title compound with is purified by chromatography on silica **gel**.

DETD . . . stirred at room temperature for 5 days, volatiles are removed in vacuo. The product is isolated by chromatography on silica **gel** as described in Example 98.

DETD . . . 172 and then treated with benzoic acid instead of morpholine, whereupon the mixture is heated. Purification by chromatography on silica **gel** provides the title compound.

DETD . . . of the ice and is stirred for 5 days. The reaction is diluted in diethyl ether and poured onto silica **gel** (70-230 mesh, 20 mL) and allowed to air dry. The adsorbed silica is layered on fresh silica (70-230 mesh, 100. . .

DETD . . . the ice and is stirred for 5 days. The reaction is diluted in diethyl ether (25 mL), poured onto silica **gel** (70-230 mesh, 40 mL) and allowed to air dry. The adsorbed silica is layered on fresh silica (70-230 mesh, 200. . .

DETD . . . The mixture is warmed to ambient temperature and stirred for 5

days. Purification of the mixture by chromatography on silica **gel** provides the title product.

DETD . . . temperature over 8 hours and is stirred for an additional 5 hours. Purification of the mixture by chromatography on silica **gel** provides title product.

DETD . . . of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dennatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoirmnune and otherwise). . .

DETD . . . a pharmaceutically acceptable carrier or excipient, which may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, **ointments**, drops or transdermal patch), bucally, or as an oral or nasal spray. The phrase "pharmaceutically acceptable carrier" means a non-toxic. . .

DETD **Topical** administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for **topical** administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized. . .

DETD A further form of **topical** administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as automimmue diseases, allergic. . . aqueous humor, vitreous humor, cornea, iris/cilary, lens, choroid/retina and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an **ointment**, vegetable oil or an encapsulating material.

L9 ANSWER 46 OF 68 USPATFULL

AB T cell-mediated diseases in mammals are treated using compositions comprising a polycyclic aromatic compound, preferably hypericin or pseudohypericin, and related compounds, including isomers, analogs, derivatives, salts, or ion pairs of hypericin or pseudohypericin. The above composition may be administered in combination with an immunosuppressive agent. Pharmaceutical compositions useful for treating a T cell-mediated disease comprise the above polycyclic aromatic compound, alone or in combination with an immunosuppressive agent. The compositions and methods are useful in treating diseases which include multiple sclerosis, myasthenia gravis, scleroderma, polymyositis, graft-versus-host disease, graft rejection, Graves disease, Addison's disease, autoimmune uveoretinitis, autoimmune thyroiditis, **pemphigus vulgaris** and rheumatoid arthritis. Psoriasis and systemic lups erythematosus. Also provided are methods for diminishing the expression of CD4 Molecules on the surface of a T lymphocyte, and for inducing multidrug resistance in a cell, comprising incubating the cell with an effective concentration of a polycyclic aromatic compound.

AN 96:38936 USPATFULL

TI Methods and polycyclic aromatic compound containing compositions for treating T-cell-mediated diseases

IN Meruelo, Daniel, Scarborough, NY, United States
Lavie, Gad, Tenafly, NJ, United States

PA New York University, New York, NY, United States (U.S. corporation)

PI US 5514714 19960507 <--

AI US 1993-39790 19930330 (8)

RLI Continuation-in-part of Ser. No. US 1991-784952, filed on 1 Nov 1991, now abandoned which is a continuation-in-part of Ser. No. US 1990-572085, filed on 23 Aug 1990, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Henley, III, Raymond

LREP Browdy and Neimark

CLMN Number of Claims: 6

ECL Exemplary Claim: 1

DRWN 15 Drawing Figure(s); 9 Drawing Page(s)

LN.CNT 1179

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5514714 19960507 <--

AB . . . which include multiple sclerosis, myasthenia gravis, scleroderma, polymyositis, graft-versus-host disease, graft rejection, Graves disease, Addison's disease, autoimmune uveoretinitis, autoimmune thyroiditis, **pemphigus vulgaris** and rheumatoid arthritis. Psoriasis and systemic lups erythematosus. Also provided are methods for diminishing the expression of CD4 Molecules on. . .

SUMM . . . autoimmune diseases also involve administration of drugs which non-specifically suppress the immune response. Examples of such drugs include methotrexate, cyclophosphamide, **azathioprine**, FK-506 and cyclosporin A. Glucocorticosteroids, such as prednisone and methylprednisolone are also commonly employed to treat autoimmunity. These drugs have. . .

SUMM . . . method include at least one of asteroid, cyclosporin A or analogs thereof, cyclophosphamide, methotrexate, rapamycin, prednisone8 methylprednisolone, OKT-3, FK-506, 15-deoxyspergualin, **azathioprine**, anti-CD-3 monoclonal antibodies, or mixtures thereof.

SUMM . . . consisting of multiple sclerosis, myasthenia gravis, scleroderma, polymyositis, graft-versus-host disease, graft rejection, Graves disease, Addison's disease, autoimmune uveoretinitis, autoimmune thyroidiris, **Pemphigus vulgaris**, systemic lupus erythematosus, primary biliary cirrhosis, rheumatoid arthritis. In a preferred embodiment the method is used to prevent or treat. . .

SUMM . . . at least one of cyclosporin A, cyclophosphamide, methotrexate, a steroid, rapamycin, an anti-CD3 monoclonal antibody, prednisone, methylprednisolone, OKT-3, FK-506, 15-deoxyspergualin, **azathioprine** and mixtures thereof. A preferred composition further comprises a pharmaceutically-acceptable carrier or diluent.

DRWD . . . is a graph showing the effect of hypericin administered 3 times a week on survival of mice with graft-versus-host disease (**GVHD**).

DRWD FIG. 2 is a graph showing the effect of hypericin on **GVHD** morbidity in mice treated 3 times a week with hypericin,

DRWD . . . a comparison of the efficacy of one, two, three, four or five weekly administrations of hypericin in the treatment of **GVHD** in mice.

DRWD . . . showing the effects of cyclosporin A, hypericin and the hypericin analogs WIS-3, WIS-6 and WIS-7 on morbidity of mice with **GVHD**.

DRWD . . . hypericin and the hypericin analogs WIS-3 (desmethyl-hpericin), WIS-6 (hypericin diacetate) and WIS-7 (dihydroxy desmethyl hypericin) on survival of mice with **GVHD**.

DETD . . . disease, Graves' disease, scleroderma, polymyositis, insulin dependent diabetes mellitus, autoimmune uveoretinitis, systemic lupus

erythematosus, inflammatory bowel disease including ulcerative colitis, **pemphigus vulgaris**, autoimmune thyroiditis, primary biliary cirrhosis, psoriatic arthritis, exfoliative psoriatic dermatitis, postular psoriasis, autoimmune hemolytic anemia, mixed connective tissue disease, autoimmune. . .

DETD . . . involves administration of the composition prior to the induction of the disease. Thus, for example, in an animal model of **GVHD**, successful administration of composition prior to grafting results in "prevention" of the disease.

DETD . . . the composition after the inductive event but prior to the clinical appearance of the disease. Again, in the example of **GVHD**, successful administration of a composition after injection of the graft cells but prior to the appearance of clinical symptoms comprises. . .

DETD "Treatment" involves administration of the composition after the appearance of the disease. In the **GVHD** example, successful administration of a composition after injection of the grafted cells and after clinical signs have developed comprises "treatment". . .

DETD . . . of the immunosuppressive agents that can be used in above combinations include cyclosporin A (Sandoz Pharmaceuticals, East Hanover, N.J.), Imuran (**azathioprine**, Burroughs Welcome, Research Triangle Park, N.C.), Cytoxan, (cyclophosphamide, Bristol Meyers Oncology, Evansville, Ind.), prednisone (Lederle Laboratories, Wayne, N.J.), methylprednisolone (Duramed. . .

DETD . . . between about 1 and 20 mg/kg body weight per day for treating kidney graft rejection in humans. In like manner, **azathioprine** (Imuran.TM.) can be used at dosages broadly ranging between about 0.1 and 20 mg/kg body weight per day, while prednisone. . .

DETD . . . using suppositories for use in treating mammals that are afflicted with T cell-mediated diseases. Topically by incorporation into skin penetrating **creams** or propylene-glycol, or other **creams** which are absorbed into the deep layers of the skin. The pharmaceutical formulations of the invention comprise an effective amount. . .

DETD . . . in the art (e.g. suppositories) are also contemplated for use in administering the active ingredients of the present invention or **creams** containing Hy or analogs thereof for **topical** administration.

DETD . . . be either in sprayable or nonsprayable form. Non-sprayable forms can be semi-solid or solid forms comprising a carrier conducive to **topical** application and having a dynamic viscosity preferably greater than that of water. Suitable formulations included but are not limited to, solution, suspensions, emulsions, **creams**, **ointments**, powders, liniments, salves, and the like. If desired, these may be sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers, or salts for influencing osmotic pressure and the like. Preferred vehicles for non-sprayable **topical** preparations include **ointment** bases, e.g. polyethylene glycol-1000 (PEG-1000), conventional **creams** such as HEB **cream**; **gels**; as well as petroleum jelly and the like.

DETD Suitable formulations for **topical** administration include **creams**, **gels**, jellies, mucilages, pastes and **ointments**. The compounds may also be formulated for transdermal administration, for example, in the form of transdermal patches so as to. . .

DETD Also suitable for systemic or **topical** application, in

particular to the mucus membranes and lungs, are sprayable aerosol preparations wherein the active ingredient, preferably in combination. . .

DETD **GVHD** in mice is a well recognized model of a T cell-mediated disease. C3H/DiSN mice which have the MHC type H-2.sup.k. . .

DETD . . . of morbidity and survival. At a cell inoculum of 5.times.10.sup.6 cells/mouse, all of the control mice began manifesting symptoms of **GVHD** as early as day 16 after transplantation and by day 35, all of the control mice were affected (Table 1).. . . 150 .mu.g hypericin remained healthy throughout the entire experiment. When a large inoculum of grafted cells was used to induce **GVHD** (2.times.10.sup.7 cells) , hypericin was not effective at 150 .mu.g/mouse.

DETD These results establish that hypericin was effective in lessening the symptoms of **GVHD** and prolonging the survival of treated animals.

DETD Effect of Different Frequencies of Hypericin Administration on Acute **GVHD**

DETD . . . results show that hypericin treatment, at a frequency of four and five times per week was more effective in treating **GVHD** than a three injections per week regimen.

DETD Effects of Hypericin Analogs and Derivatives on **GVHD**

DETD . . . of three hypericin analogs and derivatives, hypericin diacetate (WIS-6), desmethyl hypericin (WIS-3) and dihydroxydesmethyl hypericin (WIS-7) were tested in the **GVHD** system. These treatments were compared with hypericin or cyclosporin A (Sandoz Pharmaceuticals, East Hanover, N.J.), one of the most effective. . .

DETD As is shown in Table 2, mice in the control groups (irradiation and cells only) began manifesting symptoms of **GVHD** as early as 15 days post-transplantation. Three of four were dead by day 28. WIS-3 and WIS-6 may have had a small effect in ameliorating **GVHD** symptoms. In contrast, 2 of 3 hypericin-treated mice were healthy and showed no symptoms of **GVHD** throughout the entire 46 day follow-up period.

DETD . . . cyclosporin A. Both hypericin diacetate (WIS-6) and dihydroxydesmethyl hypericin (WIS-7) appeared to have a small effect in preventing or ameliorating **GVHD**, compared to the no drug group.

DETD These results show that hypericin, WIS-7 (and perhaps WIS-3) were more effective in treating **GVHD** than was cyclosporin A.

CLM What is claimed is:

. . . consisting of multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, scleroderma, polymyositis, Graves disease, Addison's disease, psoriasis, autoimmune uveoretinitis, autoimmune thyroidiris, **Pemphigus vulgaris** and rheumatoid arthritis.

L9 ANSWER 47 OF 68 USPATFULL

AB Novel ruthenium complexes for use as immunosuppressive agents to prevent or significantly reduce graft rejection in organ and bone marrow transplantation are described. The ruthenium complexes can also be used as an immunosuppressant drug for T-lymphocyte mediated autoimmune diseases, such as diabetes, and may be useful in alleviating psoriasis and contact dermatitis.

AN 96:36681 USPATFULL

TI Compounds for inhibiting immune response

IN Bastos, Cecilia M., Westborough, MA, United States

PA Procept, Inc., Cambridge, MA, United States (U.S. corporation)

PI US 5512687 19960430 <--
AI US 1994-331388 19941028 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Richter, Johann; Assistant Examiner: Cross, Laura R.
LREP Hamilton, Brook, Smith & Reynolds
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 306
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5512687 19960430 <--
SUMM . . . dosage formulations containing a physiologically acceptable vehicle and optional adjuvants and preservatives. Suitable physiologically acceptable vehicles include saline, sterile water, **creams, ointments** or solutions.
SUMM Ruthenium complexes can be applied topically as a **cream** or **ointment** to locally deliver immunosuppressive concentrations of the drug without significant systemic exposure. **Topical** application may be the ideal way to deliver the compound in psoriasis and perhaps other inflammatory skin diseases such as contact dermatitis and **pemphigus vulgaris.**
SUMM . . . immunosuppressive effect. Compounds that can be coadministered include steroids (e.g. methyl prednisolone acetate), NSAIDS and other known immunosuppressants such as **azathioprine,** 15-deoxyspergualin, cyclosporin, mizoribine, mycophenolate mofetil, brequinar sodium, leflunomide, FK-506, rapamycin and related molecules. Dosages of these drugs will also vary. . .

L9 ANSWER 48 OF 68 USPATFULL
AB A class of 2,6-diarylpyridazinones of general structural formula I have been identified that exhibit exhibit immunosuppressant activity with human T-lymphocytes, and are useful as an immunosuppressants. ##STR1## or a pharmaceutically acceptable salt, hydrate or crystal form thereof, wherein:

when M is S, R.sup.1 and R.sup.2 are selected from the following combinations:

when R.sup.2 is 4-chloro, then R.sup.1 is 4-OCH.sub.3,2 -CH.sub.3, 4-Cl,
4-CH3,

3-Cl, 3-CH3, 2-Cl, 4-H, 4-Br, 3-NO.sub.2 ; and

when R.sup.2 is H, then R.sup.1 is 4-OCH.sub.3, and

when M is --SO.sub.2 --, then R.sup.2 is H and R.sup.1 is 4-OCH.sub.3.

As an immunosuppressant, these compounds are useful in the treatment of autoimmune diseases, the prevention of rejection of foreign organ transplants and/or related afflictions, diseases and illnesses.
AN 96:29557 USPATFULL
TI 2,6-diaryl pyridazinones with immunosuppressant activity
IN Norton, Richard, Somerset, NJ, United States
Ibraham, Mohammed K. A., Imbaba-Giza, Egypt
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5506228 19960409 <--
AI US 1995-392580 19950223 (8)
DT Utility

FS Granted
EXNAM Primary Examiner: Daus, Donald G.
LREP Bigley, Francis P., Daniel, Mark R.
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 805
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5506228 19960409 <--
SUMM . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, Graves ophthalmopathy and asthma.
SUMM . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne, Alopecia areata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .
SUMM . . . parenteral applications. The active ingredient may be compounded, for example, with the usual non- toxic, pharmaceutically acceptable carders for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .
SUMM . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of: **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester, cyclosporin, FK-506 and rapamycin.
DETD . . . residue was dissolved in n-hexane:ethyl acetate (2:1) (approximately 400 ml) and the solution was passed over 1000 g of silica **gel**. Elution with n-hexane:ethyl acetate (3:1) yielded 11.66 g of 1-chloro- 1-[(4-methoxyphenyl)hydrazono]-2-propanone, mp 114.degree.-116.degree. C. (hexane).
DETD . . . of 1-chloro-1-[(4-methoxyphenyl)hydrazono]-2-propanone. The purity of the product was sufficient for further utilization. Further purification was accomplished by chromatography over silica **gel** and elution with elution with n-hexane:ethyl acetate (3:1) to yield 1-chloro-1-[(4 -methoxyphenyl)hydrazono]-2-propanone, mp 114.degree.-116.degree. C. (hexane).
DETD . . . (GIBO). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO) for 2 minutes at 4.degree. C. Cold medium was added and cells were again. . .
CLM What is claimed is:
4. The pharmaceutical formulation of claim 3, comprising in addition, an antiproliferative agent selected from the group consisting of: **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester, cyclosporin, FK-506 and rapamycin.

7. The method of claim 6, wherein the antiproliferative agent is selected from the group consisting of **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester,

cyclosporin FK-506 and rapamycin.

L9 ANSWER 49 OF 68 USPATFULL
AB This invention relates to the use of Ruthenium Red as an immunosuppressive agent to prevent or significantly reduce graft rejection in organ and bone marrow transplantation. Ruthenium Red can also be used as an immunosuppressant drug for T lymphocyte mediated autoimmune diseases, such as diabetes. Furthermore, Ruthenium Red may be useful in alleviating psoriasis and contact dermatitis.
AN 96:10989 USPATFULL
TI Method for suppressing immune response associated with psoriasis, contact dermatitis and diabetes mellitus
IN Dwyer, Donard S., Shreveport, LA, United States
Esenther, Kristin, Ashland, MA, United States
PA Procept, Inc., Cambridge, MA, United States (U.S. corporation)
PI US 5489441 19960206 <--
AI US 1993-109232 19930819 (8)
RLI Continuation-in-part of Ser. No. US 1992-817536, filed on 7 Jan 1992, now patented, Pat. No. US 5238689
DT Utility
FS Granted
EXNAM Primary Examiner: Cook, Rebecca
LREP Hamilton, Brook, Smith & Reynolds
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 515
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5489441 19960206 <--
DETD The effects of **topical** application in mice suggest that in humans also, **topical** application of Ruthenium Red in a **cream** or **ointment** could deliver locally immunosuppressive concentrations of the drug without significant systemic exposure. **Topical** application may be the ideal way to deliver the compound in psoriasis and perhaps other inflammatory skin diseases such as contact dermatitis and **pemphigus vulgaris**. Herein are described experiments which demonstrate in vitro that Ruthenium Red can penetrate human skin sufficiently to achieve T cell. . .
DETD . . . dosage formulations containing a physiologically acceptable vehicle and optional adjuvants and preservatives. Suitable physiologically acceptable vehicles include saline, sterile water, **creams**, **ointments** or solutions.
DETD . . . immunosuppressive effect. Compounds that can be coadministered include steroids (e.g. methyl prednisolone acetate), NSAIDS and other known immunosuppressants such as **azathioprine**, 15-deoxyspergualin, cyclosporin and related molecules. Dosages of these drugs will also vary depending upon the condition and individual to be. . .
DETD . . . erythema at the site of exposure. The immunological response was predominantly due to T lymphocytes. The treated mice then received **topical** administration of Ruthenium Red in petrolatum (at a 0.5, 1 or 2% final concentration) 1 hours and 12 hours later.. . . topically, the data also indicate transdermal absorption of the material which is a critical requirement for therapy of psoriasis by **topical** application of compound.
CLM What is claimed is:

3. The method of claim 1 wherein the Ruthenium Red is administered as a **cream, ointment** or solution.

8. The method of claim 6 wherein the Ruthenium Red is administered as a **cream, ointment** or solution.

L9 ANSWER 50 OF 68 USPATFULL
AB The present invention relates to intercellular adhesion molecules (ICAM-1) which are involved in the process through which lymphocytes recognize and migrate to sites of inflammation as well as attach to cellular substrates during inflammation. The invention is directed toward such molecules, screening assays for identifying such molecules and antibodies capable of binding such molecules. The invention also includes uses for adhesion molecules and for the antibodies that are capable of binding them.
AN 95:110539 USPATFULL
TI R6-5-D6, an antibody which binds intercellular adhesion molecule-1
IN Springer, Timothy A., Newtown, MA, United States
Rothlein, Robert, Danbury, CT, United States
Marlin, Steven D., Danbury, CT, United States
Dustin, Michael L., University City, MO, United States
PA The Dana Farber Cancer Institute, Boston, MA, United States (U.S. corporation)
PI US 5475091 19951212 <--
AI US 1994-186457 19940125 (8)
RLI Division of Ser. No. US 1990-515478, filed on 27 Apr 1990, now patented,
Pat. No. US 5284931 which is a continuation-in-part of Ser. No. US 1987-45963, filed on 4 May 1987, now abandoned And a continuation-in-part of Ser. No. US 1987-115798, filed on 2 Nov 1987, now abandoned Ser. No. Ser. No. US 1988-155943, filed on 16 Feb 1988, now abandoned Ser. No. Ser. No. US 1988-189815, filed on 3 May 1988, now
abandoned Ser. No. Ser. No. US 1988-250446, filed on 28 Sep 1988, now abandoned Ser. No. Ser. No. US 1989-324481, filed on 16 Mar 1989, now abandoned Ser. No. Ser. No. US 1989-373882, filed on 19 Jun 1989, now abandoned And Ser. No. US 1989-456647, filed on 22 Dec 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Chan, Christina Y.
LREP Sterne, Kessler, Goldstein & Fox
CLMN Number of Claims: 2
ECL Exemplary Claim: 1,2
DRWN 33 Drawing Figure(s); 25 Drawing Page(s)
LN.CNT 5026
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5475091 19951212 <--
SUMM (b) at least one immunosuppressive agent selected from the group consisting of: dexamethesone, **azathioprine** and cyclosporin A.
DETD . . . such a screen. Thus, for example, the antigen bound by the antibody may be analyzed as by immunoprecipitation and polyacrylamide **gel** electrophoresis. If the bound antigen is a member of the LFA-1 family of molecules then the immunoprecipitated antigen will be. . .
DETD . . . a TEFLON POTTER ELVEJHEM homogenizer, and then centrifuged at 1000.times. g for 15 minutes. The supernatant was retained and the **pellet** was re-extracted with 200 ml of 2.5% TWEEN 40 in Tris-saline. After centrifugation at 1000.times. g for 15 minutes, the

supernatants from both extractions were combined and centrifuged at 150,000.times. for 1 hour to **pellet** the membranes. The membranes were washed by resuspending in 200 ml Tris-saline, centrifuged at 150,000.times. for 1 hour. The membrane **pellet** was resuspended in 200 ml Tris-saline and was homogenized with a motorized homogenizer and TEFLON pestle until the suspension was. . .

DETD . . . be used in structural studies, a column of 10 ml of RR1/1-SEPHAROSE CL-4B (coupled at 2.5 mg of antibody/ml of **gel**), and two 10 ml pre-columns of CNBr-activated, glycine-quenched SEPHAROSE CL-4B, and rat-IgG coupled to SEPHAROSE CL-4B (2 mg/ml) were used.. . .

DETD Approximately 200 .mu.g of purified ICAM-1 was subjected to a second stage purification by preparative SDS-polyacrylamide **gel** electrophoresis. The band representing ICAM-1 was visualized by soaking the **gel** in 1M KCl. The **gel** region which contained ICAM-1 was then excised and electroeluted according to the method of Hunkapiller et al., Meth. Enzymol. 91:227-236. . .

DETD ICAM-1 purified from human spleen migrates in SDS-polyacrylamide **gels** as a broad band of M.sub.r of 72,000 to 91,000. ICAM-1 purified from JY cells also migrates as a broad. . .

DETD . . . to Eco R1 linkers (New England Biolabs), digested with Eco R1 and size selected on a low melting point agarose **gel**. cDNA greater than 500 bp were ligated to .lambda.gt10 which had previously been Eco R1 digested and dephosphorylated (Stratagene) The. . .

DETD . . . the manufacturers recommended quantity of Bam H1 and Eco R1 endonucleases (New England Biolabs). Following electrophoresis through a 0.8% agarose **gel**, the DNAs were transferred to a nylon membrane (Zeta Probe, BioRad). The filter was prehybridized and hybridized following standard procedures. . . 20 .mu.g of total RNA or 6 .mu.g of poly(A).sup.+ RNA. RNA was denatured and electrophoresed through a 1% agarose-formaldehyde **gel** and electrotransferred to Zeta Probe. Filters were prehybridized and hybridized as described previously (Staunton, D. E., et al. Embo J.. . .

DETD . . . diseases were studied for their expression of ICAM-1 and HLA-DR. A proportion of keratinocytes in biopsies of allergic contact eczema, **pemphigoid**/pemphigus and lichen planus expressed ICAM-1. Lichen planus biopsies showed the most intense staining with a pattern similar to or even. . .

DETD . . . Diseases

Diagnosis	No. of Cases	ICAM-1 Only	HLA-DR Only	ICAM-1 & HLA-DR
Allergic Contact Eczema	5	3.sup.a	0	2
Lichen Planus	11	3	0	8
Pemphigoid/ Pemphigus	2	2	0	0
Exanthema	3	2	0	0
Urticaria	4	1	0	1

.sup.a Samples were considered as positive if at. . .

DETD . . . and anti-LFA-1 antibodies. In order to determine whether the combined administration of anti-ICAM-1 and other immunosuppressive agents (such as dexamethasone, **azathioprine**, cyclosporin A or steroids (such as, for example, prednisone, etc.) would also have enhanced effects, MLR assays were performed using. . .

DETD . . . the inhibitory effects of R6-5-D6 are at least additive with the inhibitory effects of suboptimal doses of dexamethasone (Table 19), **Azathioprine** (Table 20) and cyclosporin A (Table 21). This implies that anti-ICAM-1 antibodies can be effective in lowering the necessary doses. . .

DETD TABLE 20

Effect of Anti-ICAM-1 and **Azathioprine** on the Human MLR

Group	Inhibitor (ng/ml)	.sup.3 HT Incorporation (CPM)	% Inhibition
Media	-	78	-
Stimulators (S)	-	174	-
Responders (R)	-	3,419	-
R .times. S	-	49,570	-
R .times. S	R6-5-D6 (8)	44,374	11
R .times. S	**Azathioprine** (1)	42,710	14
R .times. S	R6-5-D6 (8) + **Azathioprine** (1)	34,246	31

L9 ANSWER 51 OF 68 USPATFULL

AB Immunomodulatory macrocyclic compounds having the formula ##STR1## and pharmaceutically acceptable salts, esters, amides and prodrugs thereof, wherein X is selected from one of the formulae ##STR2## as well as pharmaceutical compositions containing the same.

AN 95:90535 USPATFULL

TI Macrocyclic immunomodulators

IN Luly, Jay R., Libertyville, IL, United States
Kawai, Megumi, Libertyville, IL, United States
Or, Yat S., Libertyville, IL, United States
Wiedeman, Paul, Libertyville, IL, United States
Wagner, Rolf, Gurnee, IL, United States

PA Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

PI US 5457111 19951010 <--

AI US 1993-149416 19931109 (8)

RLI Continuation-in-part of Ser. No. US 1993-32958, filed on 17 Mar 1993, now abandoned which is a continuation-in-part of Ser. No. US 1991-755208, filed on 5 Sep 1991, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Bond, Robert T.

LREP Danckers, Andreas M., Crowley, Steven R.

CLMN Number of Claims: 12

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 7685

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5457111 19951010 <--

SUMM . . . would be useful when used alone, combination therapy with other

immunosuppressants, such as, FK506, rapamycin, cyclosporin A, picibanil, mycophenolic acid, **azathioprine**, prednisolone, cyclophosphamide, brequinar and leflunomide, would also be expected to be beneficial.

SUMM . . . of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous **pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise). . .

SUMM . . . a pharmaceutically acceptable carder or excipient, which may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, **ointments**, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carder" is meant a non-toxic. . .

SUMM **Topical** administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for **topical** administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized. . .

SUMM A further form of **topical** administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as autoimmune diseases, allergic. . . aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an **ointment**, vegetable oil or an encapsulating material.

DETD . . . and stirred at room temperature for 30 min. The solvent was removed, and the crude product was purified by silica **gel** column chromatography, eluting with 0.5%-methanol in chloroform to yield 2.043 g of the title compound. A small amount (100 mg). . .

DETD . . . dried over magnesium sulfate. Evaporation of the solvent gave 837 mg of crude product. This was purified twice by silica **gel** column chromatography, eluting with 0.5%-methanol in chloroform. Yield: 165 mg. MS (FAB) m/z: M+K=888; IR(KBr) 3440, 2960, 2930, 2880, 2820,. .

DETD . . . 10%-KHSO.sub.4, brine, 10%-NaHCO.sub.3, brine, and then dried over magnesium sulfate. The crude product (234 mg) obtained was purified by silica **gel** column chromatography, eluting with 0.5-1.5% methanol in chloroform. Yield: 68.7 mg. MS (FAB) m/z: M+K=871; IR(KBr) 3430, 2960, 2940, 2870,. . .

DETD . . . mentioned in Example 4, except benzoyl chloride (44. 1 mL, 0.30 mmol) was employed instead of acetyl chloride. After silica **gel** column chromatography, a white powder was obtained. Yield: 46.0 mg. MS (FAB) m/z: M+K=933; IR(KBr) 3440, 2960, 2940, 2880, 2830,. . .

DETD . . . anhydrous magnesium sulfate. Evaporation of the solvent gave 438 mg of the crude title compound. This was purified by silica **gel** column chromatography, eluting with 2.5% ethyl acetate in chloroform. Yield: 225.8 mg. MS (FAB) m/z: M+K=942; IR(KBr) 3500, 3440, 2950,. . .

DETD . . . anhydrous magnesium sulfate. After filtration, the filtrate was

evaporated to dryness, and the crude product obtained was purified by silica **gel** column chromatography, eluting with 10%-ethyl acetate in chloroform. Yield: 1.86 g. MS (FAB) m/z: M+K=940; IR(KBr) 3500, 2960, 2940, 2870,. . .

DETD . . . dried over anhydrous magnesium sulfate. Evaporation of the solvent gave 1.400 g of crude product which was purified by silica **gel** column chromatography, eluting with 5%-ethyl acetate in chloroform. The title compound (730 mg) was obtained. MS (FAB) m/z: M+K=966; IR(KBr). . .

DETD . . . over anhydrous magnesium sulfate. Evaporation of the solvent gave 515 mg of crude title compound which was purified by silica **gel** column chromatography, eluting with 1%-methanol in chloroform. 304.8 mg of pure compound was obtained. MS (FAB) m/z: M+K=828; IR(KBr) 3420,. . .

DETD . . . dried over anhydrous magnesium sulfate. Evaporation of the solvent yielded 920 mg of crude product. This was purified by silica **gel** column chromatography, eluting with 7%-ethyl acetate in chloroform. 648 mg of pure title compound was obtained. MS (FAB) m/z: M+K=826,. . .

DETD . . . stirring. After treating the mixture as described in Example 7,

the crude material (4.85 g) obtained was purified by silica **gel** column chromatography, eluting with 1.5% to 4%-methanol in chloroform. 184 mg of pure title compound was isolated. MS (FAB) m/z:. . .

DETD . . . is passed through a short column of Florisil to remove traces of thallium (I) bromide, concentrated, and purified on silica **gel** column chromatography to give the desired compound.

DETD . . . is added to the reaction mixture. The ethyl acetate layer is washed with brine, dried, evaporated, and purified by silica **gel** column chromatography to afford pure title compound.

DETD . . . chloride and extracted with ethyl acetate. The ethyl acetate layer is washed with brine, dried, evaporated, and purified by silica **gel** column chromatography to afford pure title compound.

DETD . . . dried over anhydrous magnesium sulfate. Evaporation of the solvent gave 1.70 g of crude product which was purified by silica **gel** (100 g) column chromatography, first eluting with 15%-acetone in hexane, followed by 30%-acetone-hexane. 290 mg of pure title compound was. . .

DETD . . . ambient temperature for 6 hours, whereupon the volatiles were removed in vacuo. The residue was purified by chromatography on silica **gel** eluting with a mixture of hexanes and acetone (4: 1) which provided the desired product (610 mg) in 75% yield.. . .

DETD . . . with ethyl acetate. The organic extracts are washed with brine,

dried, and concentrated. The residue is then purified by silica **gel** column chromatography to yield pure title compound.

DETD . . . then washed with 10%-sodium hydrogen carbonate, 10%-potassium hydrogen sulfate, brine, and dried. The crude product obtained is purified by silica **gel** column chromatography to yield pure title compound.

DETD . . . in benzene and gently refluxed for 3-5 hours. The solvent is removed, and the residue obtained is purified by silica **gel** column chromatography to yield pure title compound.

DETD . . . is washed with 10%-sodium hydrogen carbonate, brine, dried over

magnesium sulfate, and evaporated. The crude product is purified by silica **gel** column chromatography.

DETD . . . hydrogen carbonate, brine, and dried over magnesium sulfate. After the solvent is removed, the crude product is purified by silica **gel** column chromatography.

DETD . . . 10%-sodium hydrogen carbonate, brine, dried over magnesium sulfate. After the solvent is removed, the crude product is purified on silica **gel** column chromatography.

DETD . . . 10%-sodium hydrogen carbonate, brine, dried over magnesium sulfate. After the solvent is removed, the crude product is purified on silica **gel** column chromatography.

DETD . . . 10%-sodium hydrogen carbonate, brine, dried over magnesium sulfate. After the solvent is removed, the crude product is purified by silica **gel** column chromatography.

DETD . . . 10%-sodium hydrogen carbonate, brine, dried over magnesium sulfate. After the solvent is removed, the crude product is purified on silica **gel** column chromatography.

DETD . . . and then dried over anhydrous magnesium sulfate. Evaporation of the solvent gave crude product which was purified by flash silica **gel** (25 g) column chromatography, eluting 15%-acetone in hexane. 73 mg of pure title compound was isolated. MS (FAB) m/z: M+K=1062.

DETD . . . hydrogen carbonate, brine, and dried over magnesium sulfate. After the solvent is removed, the crude product is purified on silica **gel** column chromatography.

DETD . . . hydrogen carbonate, brine, and dried over magnesium sulfate. After the solvent is removed, the crude product is purified on silica **gel** column chromatography.

DETD . . . hydrogen carbonate, brine, and dried over magnesium sulfate. After the solvent is removed, the crude product is purified by silica **gel** column chromatography.

DETD . . . hydrogen carbonate, brine, and dried over magnesium sulfate. After the solvent is removed, the crude product is purified on silica **gel** column chromatography.

DETD . . . hydrogen carbonate, brine, and dried over magnesium sulfate. After the solvent is removed, the crude product is purified on silica **gel** column chromatography.

DETD . . . absolute ethanol (10 mL) was refluxed under nitrogen overnight. After removal of solvent, the solid residue was purified by silica **gel** chromatography with ether elution. Yield: 0.6 g; mp 92.degree.-98.degree. C.; MS (FAB) m/z: M+H=897, M+NH.sub.4 =914.

DETD . . . g) in absolute ethanol was refluxed under nitrogen overnight. Solvent was removed in vacuo and the product purified by silica **gel** chromatography (10 g) with methylene chloride/acetonitrile (5:2, v/v) elution, followed by ether. Yield: 0.3 g; MS (FAB) m/z: M+H=846, M+NH.sub.4. . .

DETD . . . absolute ethanol (3 mL) was refluxed under nitrogen overnight. Solvent was removed in vacuo and the product purified on silica **gel** (10 g) with methylene chloride/acetonitrile (5:2, v/v) elution, followed by 40% acetone in hexanes. Yield: 0.396 g; mp 110.degree.-120.degree. C.;. . .

DETD . . . with methylene chloride. The solvent was removed in vacuo and residue solid was redissolved in methylene chloride, filtered through silica **gel** eluting with 50% acetone in hexanes, and concentrated in vacuo. The solid was recrystallized from ether-hexanes. Yield: 4.9 g; mp. . .

DETD . . . ethanol (3 mL) was refluxed under nitrogen overnight. Solvent was removed in vacuo, and the product was purified on silica **gel** with ether elution. Yield: 0.23 g; mp: 133.degree.-138.degree. C.; MS (FAB) m/z: M+H=881.

DETD . . . (aq) (2.times.30 mL), saturated brine (30 mL), dried over magnesium sulfate and solvent removed. The product was purified on silica **gel** (70 g) with ether elution. Yield: 0.95 g; MS (FAB) m/z: M+H=790.

DETD . . . ethanol was refluxed under nitrogen for 1.5 hours. Solvent was removed in vacuo and the product was purified on silica **gel** with ether elution. Yield: 0.2 g; MS (FAB) m/z: M+H=805.

DETD . . . hours, the reaction mixture was refluxed for 2 hours. After removal of the solvent, the product was purified by silica **gel** chromatography (silica **gel**, 50 g) eluting with 50% acetone in hexanes. The solid was further purified by prep TLC (5% methanol in methylene. . .

DETD . . . was extracted with anhydrous ether (4.times.50 mL). Ether was removed in vacuo and the solid residue was purified by silica **gel** chromatography eluting with 5% acetone in hexanes providing the title compound (17 g). MS (FAB)m/z: M+H=1022.

DETD . . . and extracted with additional methylene chloride (3.times.50 mL). Solvent was removed in vacuo, and the solid residue filtered through silica **gel** (20 g) and eluted with ether. Yield: 5 g; MS (FAB) m/z: M+H=1024.

DETD . . . hour. Solvent and excess acetic anhydride is removed in vacuo (0.1 torr) and the solid residue is purified by silica **gel** chromatography eluting with 2% ethanol in methylene chloride.

DETD . . . and 1N hydrochloric acid. The organic phase was dried over magnesium sulfate and solvent removed in vacuo. Purification by silica **gel** chromatography eluting with 10% ether in hexanes provided the title compound (9 g). MS (FAB) m/z: M+Na=1128.

DETD . . . and the solid was filtered off and extracted with methylene chloride (3.times.50 mL). The resulting solution was filtered through silica **gel** (20 g) and eluted with ether. Yield: 5 g; MS (FAB) m/z: M+Na=1130.

DETD . . . hour. Solvent and excess acetic anhydride was removed in vacuo (0.1 torr) and the solid residue was purified by silica **gel** chromatography eluting with 2% ethanol in methylene chloride. Yield: 1 g; MS (FAB) m/z: M+Na=1172.

DETD . . . ethyl acetate (3.times.50 mL), dried over magnesium sulfate and the solvent removed in vacuo. The crude is purified by silica **gel** chromatography using 5% methanol in methylene chloride as eluant.

DETD . . . is refluxed under nitrogen for 16 hours. Solvent is removed in vacuo and the solid residue is purified by silica **gel** chromatography eluting first with 20% acetonitrile in methylene chloride followed by 7% methanol in methylene chloride.

DETD . . . is refluxed under nitrogen for 16 hours. Solvent is removed in vacuo and the solid residue is purified by silica **gel** chromatography eluting first with 20% acetonitrile in methylene chloride followed by 7% methanol in methylene chloride.

DETD . . . is refluxed under nitrogen for 24 hours. Solvent is removed in vacuo, and the solid residue is purified by silica **gel** chromatography eluting with 10% methanol in methylene chloride.

DETD . . . is refluxed under nitrogen for 24 hours. Solvent is removed in vacuo and the solid residue is purified by silica **gel** chromatography eluting with 10% methanol in methylene chloride.

DETD . . . The combined organic phase is washed with water, brine and dried over magnesium sulfate. The product is purified by silica **gel** chromatography eluting with 3% methanol in methylene chloride.

DETD . . . acetate (3.times.50 mL), dried over magnesium sulfate and the solvent is removed in vacuo. The crude is purified by silica **gel** chromatography using 5% methanol in methylene chloride as eluant.

DETD . . . for 24 hours. The solid is filtered off, and solvent is removed

in vacuo. The product is purified by silica **gel** chromatography eluting with 3% methanol in methylene chloride.

DETD . . . The combined organic phases are washed with water, brine and dried over magnesium sulfate. The product is purified by silica **gel** chromatography eluting with 3% methanol in methylene chloride.

DETD . . . is allowed to stir at room temperature overnight. After removal of solvent in vacuo, the product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . mixture is allowed to stir at room temperature overnight. After removal of solvent, the bis-silylated product is purified by silica **gel** chromatography and is deprotected according to the procedure of Example 60.

DETD . . . (5 mL) is heated at 80.degree. to 90.degree. C. for 12 hours. Solvent is removed and product purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . mL) is heated at 80.degree. to 90.degree. C. for 12 hours. Solvent is removed, and the products purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . is heated at 80.degree. to 90.degree. C. for 12 hours. Solvent is removed, and the products are purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . (5 mL) is heated at 80.degree. to 90.degree. C. for 12 hours. Solvent is removed and products purified by silica **gel** chromatography eluting with 20% methanol in methylene chloride.

DETD . . . is extracted with anhydrous ether (4.times.50 mL). Ether is removed in vacuo and the solid residue is purified by silica **gel** chromatography eluting with 5% acetone in hexanes.

DETD . . . at -78.degree. C. for 2 hours and worked up with saturated ammonium chloride and ether. Product is purified by silica **gel** chromatography eluting with ether.

DETD . . . 0.degree. C. for 4 hours and worked up with saturated ammonium chloride and ether. The product is purified by silica **gel** chromatography eluting with ether.

DETD . . . organic phase is dried over magnesium sulfate, and the solvent is removed in vacuo. The residue is purified by silica **gel** chromatography eluting with 20% acetone in hexanes.

DETD . . . stirring at room temperature for 26 hours, the solvent is removed in vacuo and the residue is purified by silica **gel** chromatography eluting with 10% acetone in hexanes.

DETD . . . washed with brine, dried over magnesium sulfate and the solvent is removed in vacuo. The residue is purified by silica **gel** chromatography eluting with 20% acetone in hexanes.

DETD . . . tri-n-butyltin hydride (1 mL) over 0.5 hours. The solvent is removed in vacuo and the residue is purified by silica **gel** chromatography eluting with 10% acetone in hexanes.

DETD . . . off and triturated with methylene chloride (3.times.50 mL). Solvent is removed in vacuo, and the residue is purified with silica **gel** chromatography (10 g) eluting with ether.

DETD . . . dried over sodium sulfate and freed of solvent. The title compounds were separated and purified by flash chromatography on silica **gel** using a step gradient of 15-30% acetone/hexane in steps of 5% to furnish the two products in yields of 355. . .

DETD . . . drying over sodium sulfate. The solvent was removed under reduced pressure to supply crude material which was purified by silica **gel** chromatography as described above to furnish the two products. (.sup.1 H NMR, .sup.13 C NMR, and mass spectral data

consistent. . .

DETD . . . was cooled, and the volatiles were removed under reduced pressure. The crude material was purified by flash chromatography over silica **gel** (elution with hexanes:acetone 3:2) to supply the title compounds.

DETD . . . several hours turning a darker blue throughout this time. The reaction mixture is cooled, and a small amount of silica **gel** is added prior to solvent removal under reduced pressure. The residue is applied to a silica **gel** column and the title product, the beta-hydroxy ketone, as well as the dehydration product Example 97c, are eluted with ether.

DETD . . . organic layer was dried over sodium sulfate and concentrated in vacuo. The product was purified by flash chromatography on silica **gel** using 25% acetone/hexane as eluent. Overlapped fractions were further purified by radial chromatography on silica **gel**. The title compound was obtained as a colorless foam (0.66 g). MS (FAB) m/z: M+K=965. (.sup.1 H NMR and .sup.13. . .

DETD . . . mL). The mixture was stirred for 0.5 hours. The solvent was removed, and the crude material was purified by silica **gel** column chromatography using 2% MeOH/CH.sub.2 Cl.sub.2, yielding 48 mg of the title compound. MS (FAB) m/z: M+K814.

DETD . . . dried over Na.sub.2 SO.sub.4. Evaporation of the solvent gave 94 mg of the crude material which was chromatographed over silica **gel** using 20% EtOAc/CHCl.sub.3 as an eluant. Unreacted starting material (10 mg), 18 mg of Example 103d, the silylated minor isomer.

DETD . . . and dried over Na.sub.2 SO.sub.4. The solvent was evaporated and 1.32 g of the crude material was chromatographed over silica **gel** column to give 868 mg of disilylated product and 140 mg of the recovered starting material.

DETD Aqueous HF cleavage of the resultant product of Example 104b followed by silica **gel** column purification provided 85 mg of the title compound. MS (FAB) m/z: M+K=830.

DETD . . . absolute ethanol (3 mL) is refluxed under nitrogen overnight. Solvent is removed in vacuo and product is purified on silica **gel** (10 g) with methylene chloride/acetonitrile (5:2, v/v) elution, followed by 40% acetone in hexanes to give the desired compound.

DETD . . . at 1 atm. The catalyst is filtered, the solvent is concentrated, and the resulting crude material is purified by silica **gel** column chromatography (eluting solvent, chloroform/acetone 5:1) to give the title compound.

DETD . . . and DMAP (1.5 mg) were added. After 6 hours, the mixture was evaporated, and the residue was chromatographed on silica **gel** using ether/dichloromethane (1/2) as eluant. Combination of selected fractions provided the less polar bis-acylated product Example 106b .sup.1 H NMR. . .

DETD . . . shows consumption of the resultant compound of example 105b, the solvent is evaporated, and the residue is chromatographed on silica **gel**. Combination of selected fractions provides the mono-acylated product Example 108.

DETD . . . (Na.sub.2 SO.sub.4), filtered., and concentrated in vacuo to give a yellow foam (2.21 g). The mixture was purified by silica **gel** chromatography to give the title compound (0.23 g). mp 100.degree.-105.degree. C.; IR (CDCl.sub.3) 3590, 3470, 2930, 1745,

1720, 1690, 1645,. . .

DETD . . . were stirred at ambient temperature for 16 hours and concentrated to dryness. The mixture was purified by chromatography on silica **gel** eluting with hexane/acetone mixtures to give pure title compound (3.6 g). An analytical sample was recrystallized from methylene chloride and. . .

DETD . . . washed with brine (2.times.10 mL), dried (Na.sub.2 SO.sub.4) and concentrated to dryness. The residue is purified by chromatography on silica **gel** to provide the title compound.

DETD . . . room temperature overnight, and then refluxed for 6 hours. The solvent was removed and the products were purified by silica **gel** chromatography eluting with 5% methanol in methylene chloride. Yield: 0.7 g; MS (FAB) m/z: M+K=825.

DETD . . . in absolute ethanol (11 mL) was refluxed overnight. The solvent was removed, and the intermediate product, was purified by silica **gel** chromatography. MS (FAB) m/z: M+K=932. The intermediate product (0.18 g) and glyoxal (40% in water, 0.06 g) in absolute ethanol. . . (5 mL) was heated at 50.degree. C. for 5 hours. After removal of solvent, the product was purified by silica **gel** chromatography eluting with 10% isopropanol in methylene chloride. Yield: 0.075 g; MS (FAB) m/z: M+NH.sub.4 =933.

DETD FK-506 (2 g) was oxidized according to the procedure described in Example 48. The products were purified by silica **gel** chromatography eluting with 5% acetone in hexanes. Yield: Example 159a, 0.3 g; MS (FAB) m/z: M+K=838; Example 159b, 0.9 g;. . .

DETD . . . over anhydrous sodium sulfate. Evaporation of the solvent gave 35 g of crude title compound which was purified by silica **gel** column chromatography, followed by HPLC eluting with 25%-acetone in hexane. 24.28 g (85%) of pure compound was obtained. MS (FAB). . .

DETD . . . dried over sodium sulfate. Solvent was removed to yield 2.24 g of crude product which was then purified by silica **gel** chromatography, eluting with 10% acetone in n-hexane. 800 mg of the title compound was isolated in 74% yield. MS (FAB). . .

DETD . . . was filtered and the filtrate was evaporated to dryness. The residue was re-dissolved in methylene chloride and passed through silica **gel** column, eluting with 15% acetone in n-hexane. The obtained crude product (680 mg) was finally purified by HPLC (column: microsorb,. . .

DETD . . . toluene and stirred at 70.degree. C. for one over night. Solvent was removed and the residue was purified by silica **gel** column chromatography, eluting with 5-10% acetone in hexane. 8.89 g of the title compound was isolated in 91% yield. MS. . .

DETD . . . was concentrated in vacuo to obtain the title compound in quantitative yield. The obtained product was then loaded on silica **gel** column, and eluted with 5-10% acetone in hexane to obtain the pure title compound in 80% yield. MS (FAB) m/z:. . .

DETD . . . then stirred at room temperature for 1.5 hours. Solvent was removed and the crude product obtained was purified by silica **gel** column chromatography eluting with 15% acetone in n-hexane to yield 982 mg of the product. The final purification was carried. . .

DETD . . . magnesium sulfate. After the removal of solvent, 1.45 g of the crude product was isolated. This was purified by silica **gel** column chromatography, eluting with 30% acetone in n-hexane to obtain 619 mg of the pure compound in 57% yield. MS. . .

DETD . . . (0.2 g) in absolute ethanol is refluxed under nitrogen

overnight. After removal of solvent, the product is purified by silica **gel** chromatography.

DETD . . . in absolute ethanol (10 mL) is refluxed under nitrogen overnight. After removal of solvent, the product is purified by silica **gel** chromatography.

DETD . . . once with brine, dried over magnesium sulfate and the solvent is removed in vacuo. The product is purified by silica **gel** chromatography.

DETD . . . The reaction is followed by TLC analysis. The catalyst is then filtered off, solvent removed and product purified by silica **gel** chromatography.

DETD . . . and 1N HCl. The organic phase is washed once with brine and solvent removed. The products are purified by silica **gel** chromatography.

DETD A solution containing the products of Examples 219a and 219b and silica **gel** in methylene chloride is stirred at room temperature overnight. The silica **gel** is filtered off, solvent removed and product purified by silica **gel** chromatography..

DETD . . . 1.26 mmol). The solvent was removed under reduced pressure, and

the crude material was purified by flash chromatography On silica **gel** eluting with 40% acetone in hexane. The title compound was obtained as a colorless solid (431 mg): mp 193.degree.-194.degree. C.;. . .

DETD . . . extracts were dried over magnesium sulfate and freed of solvent. The isomeric allylic alcohols were purified and separated by silica **gel** chromatography using 25% acetone in hexane as eluant. Those fractions containing pure higher and lower Rf alcohols respectively were combined. . .

DETD . . . reaction is quenched with water and extracted with ethyl acetate. Solvent is removed and the product is purified by silica **gel** chromatography.

DETD . . . mL) at room temperature. After stirring at room temperature overnight the solvent is removed and the product purified by silica **gel** chromatography.

DETD . . . is washed once with brine, dried over magnesium sulfate and the

solvent is removed. The product is purified by silica **gel** chromatography.

DETD . . . is washed once with brine, dried over magnesium sulfate, and the solvent is removed. The product is purified by silica **gel** chromatography.

DETD . . . organic phase is washed once with brine, dried over magnesium sulfate and solvent removed. The product is purified by silica **gel** chromatography.

DETD . . . stored at 0.degree. C. overnight, the reaction mixture is refluxed for an additional hour. The product is purified by silica **gel** chromatography.

DETD . . . 231 (0.4 g) and diethylacetylene dicarboxylate (1 mL) is stirred at room temperature overnight. The triazole is purified by silica **gel** chromatography.

DETD . . . in absolute ethanol (5 mL) is refluxed for 6 hours. After removal of solvent, the product is purified by silica **gel** chromatography.

DETD . . . organic phase is washed once with brine, dried over magnesium sulfate and solvent removed. The product is purified by silica **gel** chromatography.

DETD . . . organic phase is washed once with brine, dried over magnesium sulfate and solvent removed. The product is purified by silica **gel** chromatography.

DETD . . . dried over anhydrous magnesium sulfate. Evaporation of the solvent gave 364 mg of crude product which was purified by silica **gel** (50 g) column chromatography, eluting 2.5%-ethyl acetate in chloroform. Yield: 168.7 mg of pure title compound was isolated. MS (FAB). . .

DETD . . . dried over anhydrous magnesium sulfate. Evaporation of the solvent gave 98.2 mg of crude product which was purified by silica **gel** (25 g) column chromatography, eluting with 1.5%-methanol in chloroform. 63.4 mg of pure title compound was isolated. MS (FAB) m/z:. . .

DETD . . . over anhydrous magnesium sulfate. Evaporation of the solvent gave 860 mg of crude product which was purified by flash silica **gel** (120 g) column chromatography, eluting with 25%-acetone in hexane. 656 mg of pure title compound was isolated. MS (FAB) m/z:. . .

DETD . . . layers are washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The crude material is purified by silica **gel** column chromatography eluting with 20% acetone-hexane.

DETD . . . over anhydrous magnesium sulfate. Evaporation of the solvent gave 421 mg of crude product. Purification was carried out using silica **gel** column chromatography, eluting 10%-ethyl acetate in chloroform. 28 mg of pure title compound was isolated. MS (FAB) m/z: M+K=814. IR(KBr);. . .

DETD . . . min., the reaction mixture is diluted with ether and the precipitate is filtered off. The solution is filtered through silica **gel** (5 g) with ether elution. After removal of solvent, the product is purified by silica **gel** chromatography.

DETD . . . for 96 hours, and the solid is removed by filtration. After removal of solvent, the product is purified by silica **gel** chromatography.

DETD . . . added and the reaction mixture is stirred for 0.5 hours. Solvent is removed and the product is purified by silica **gel** chromatography.

DETD . . . The reaction mixture is partitioned between water and methylene chloride. After removal of solvent, the product is purified by silica **gel** chromatography.

DETD . . . with dilute hydrochloric acid, brine and dried over magnesium sulfate. After removal of solvent, the product is purified by silica **gel** chromatography.

DETD . . . C. for 1 hour, the precipitate is filtered off and solvent removed in vacuo. The product is purified by silica **gel** chromatography.

DETD . . . organic phase is washed once with brine, dried over magnesium sulfate and solvent removed. The product is purified by silica **gel** chromatography.

DETD . . . dry methylene chloride is refluxed under nitrogen for 1 hour. After removal of solvent, the product is purified by silica **gel** chromatography.

DETD . . . dried over magnesium sulfate. Solid was removed by filtration and solvent removed in vacuo. The product was purified by silica **gel** (20 g) eluting with 20% (v/v) acetone in hexanes. Yield: 0.67 g; MS (FAB) m/z: M+K=944.

DETD . . . for 1 hour. The reaction mixture was refluxed for 1 hour and solvent removed. The product was purified by silica **gel** (20 g) eluting with 20% acetone in hexanes. Yield: 0.5 g; MS (FAB) m/z: M+K=957.

DETD . . . starting material is observed. Catalytic amount of TEA is used if necessary. Solvent is removed, and is purified by silica **gel**

column chromatography to yield the title compound.

DETD . . . brine and then dried over anhydrous magnesium sulfate. Evaporation of the solvent gives crude product which is purified by silica **gel** (25 g) column chromatography, eluting 1.5%-methanol in chloroform.

DETD . . . then gently warmed until the total disappearance of starting material is observed. Solvent is removed, and is purified by silica **gel** column chromatography to yield the title compound.

C24-t-butyldimethylsilyl is removed according to the procedure described in Example 316 to give. . .

DETD . . . ethanethiol is added and stirred at room temperature for 5 hours. Solvent is removed, the residue is purified by silica **gel** column chromatography to yield the title compound.

DETD . . . absolute ethanol (3 mL) is refluxed under nitrogen overnight. Solvent is removed in vacuo and product is purified on silica **gel** (10 g) with methylene chloride/acetonitrile (5:2, v/v) elution, followed by 40% acetone in hexanes to give the desired compound.

DETD . . . Fr. 1946, 106; J. Am. Chem. Soc. 1951, 73,436). Solvent is removed in vacuo and product is purified on silica **gel** to give the desired compound.

DETD . . . ring formation (J. Am. Chem. Soc. 1986, 108, 4683). Solvent is removed in vacuo and product is purified on silica **gel** to give the desired compound.

DETD . . . 56.2 mmol) at ambient temperature (7 hours). The mixture was concentrated in vacuo and filtered through a plug of silica **gel** (300 mL, 70-230 mesh) eluting with hexane:EtOAc (1 L, 2:1). Fractions containing product were pooled and concentrated. This was purified further by HPLC on silica **gel** (50 mm.times.500 mm, 230-400 mesh) eluting with hexane:EtOAc (6 L, 5:1). The appropriate fractions were combined and concentrated to provide. . .

DETD . . . were then combined and dried (NaSO4). The solvent was removed in vacuo and the residue was passed through a silica **gel** column (300 mL, 70-230 mesh) eluting with a mixture of hexane:EtOAc (2:1, 2 L). The fractions containing product were combined and concentrated to a yellow oil (10 g) which was further purified by HPLC on silica **gel** (1 L, 230-400 mesh) eluting with hexane:EtOAc (5:1). This provided pure product (5.3 g, 6.1 mmol) in 41% yield. IR.

DETD . . . extract was decanted from the drying agent and concentrated to a colorless foam (1.04 g), which was purified by silica **gel** chromatography (70-230 mesh, 300 mL) eluting with hexane:acetone (4:1, 2.5 L). The appropriate fractions were pooled and concentrated to provide. . .

DETD . . . (2.times.30 mL). The organics were combined, dried (Na.sub.2 SO.sub.4) and concentrated in vacuo. Purification by chromatography on 70-230 mesh silica **gel** (8 g) eluting with toluene:EtOAc (5:1) provided pure product (389 mg, 0.44 mmol) as a colorless foam in 44% yield.. . .

DETD . . . for 15 minutes, when it was diluted with methylene chloride (15 mL), centrifuged and passed thru a plug of silica **gel**. The silica was eluted with hexane:acetone (1:1), and the fractions containing product were pooled, concentrated and purified by HPLC on silica **gel** eluting with hexane:acetone (2:1) providing desired product (163 mg, 0.21 mmol) in 64% yield. IR (CDCl.sub.3) 1735, 1645 cm.sup.-1. . .

DETD . . . with brine (2.times.30 mL), combined, dried (Na.sub.2 SO.sub.4)

and concentrated in vacuo. The residue was purified by HPLC on silica **gel** eluting with hexane:acetone (2.5:1) providing the title compound in 40% yield. MS (FAB) m/z 979 (M+K).

DETD . . . EtOAc (30 mL), organics were combined, dried (Na.sub.2 SO.sub.4) and concentrated in vacuo. Residue was purified by HPLC on silica **gel** eluting with hexane:acetone (1:1) to provide the title compound. MS (FAB) m/z 935 (M+K).

DETD . . . The ethyl acetate layer was washed with brine (.times.3), dried over anhydrous magnesium sulfate. Purification was carried out by silica **gel** column, followed by HPLC to obtain the title compound. Yield 159 mg (26%), MS (FAB) m/z: M+H=950, M+K=988.

DETD . . . It was then dried over anhydrous magnesium sulfate. Purification of the crude product (5.01 g) was carried out by silica **gel** column, followed by reverse phase HPLC to obtain the title compound. Yield 466 mg (17%), MS (FAB) m/z: M+K=816.

DETD . . . dried over magnesium sulfate. After deprotection according to the procedure of Example 60, the title compound was obtained by silica **gel** chromatography, followed by normal phase HPLC. Yield: 50 mg (12%); MS (FAB) m/z: M+K=812.

DETD . . . and brine, dried over Na.sub.2 SO.sub.4 and concentrated to give 0.56 g of crude product. Purification was done by silica **gel** column chromatography, eluting with 7.5% to 15% acetone in hexane. The desired product 32,24-bisTBDMS, 21-ethanol ascomycin (Example 365, 0.11 g). . .

DETD . . . using the same conditions used in Example 363. Purification of the titled bis-TBDMS protected benzoate was achieved using a silica **gel** column. Yield: 90 mg.

DETD . . . 0.63 mmol) in CH.sub.2 Cl.sub.2 (10 mL) were stirred at room temperature for 7 days. Purification was done by silica **gel** column chromatography, eluting with 5/95 acetone/hexane to give 0.31 g of 32-TBDMS, 22-S-benzyl carbonate ascomycin in 54% yield.

DETD . . . sodium sulfate, and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography on silica **gel** eluting with 40% acetone in hexane. The title compound was obtained as a solid (176 mg): mp 84.degree.-87.degree. C.; IR. . .

DETD . . . subsequently hydrogenated as described in Example 380 below to furnish the title compound after purification by flash chromatography on silica **gel** eluting with acetone and hexane.

DETD . . . subsequently hydrogenated as described in Example 380 below to furnish the title compound after purification by flash chromatography on silica **gel** eluting with acetone and hexane.

DETD . . . 38 is hydrogenated as described in Example 380 to supply the title compound after purification by flash chromatography on silica **gel** eluting with acetone and hexane.

DETD . . . falter agent and the filtrate is concentrated under reduced pressure. The crude material was purified by flash chromatography on silica **gel** eluting with 25% acetone in hexane to supply the title compound (348 mg) as a colorless foam: MS (FAB) m/e:. . .

DETD . . . The organic phase was dried and freed of solvent. The products were separated and purified by flash chromatography on silica **gel** eluting with 30% acetone in hexane. Example 381a: MS (FAB) m/e: M+K=827. Example 381b: MS (FAB) m/e: M+K=827.

DETD . . . (50 mL) and dried over magnesium sulfate. Removal of the solvent gave crude material which was flash chromatographed on silica **gel** eluting with 30% acetone in hexane. Yield 1.97 g: MS (FAB)

m/e: M+K=885; .sup.13 C NMR (75 MHz) delta (selected. . .

DETD . . . with 20 mL of brine, dried over magnesium sulfate and freed of solvent. This material was flash chromatographed on silica **gel** eluting with 30% acetone in hexane. Yield 59mg: MS (FAB) m/e: M+K=812; .sup.13 C NMR (125 MHz) delta (selected signals). . .

DETD . . . organic washes were dried over magnesium sulfate and freed of solvent. The crude material was purified by flash chromatography silica **gel** eluting with 30% acetone in hexanes. Yield 50 mg: MS (FAB) m/e: M+K=814; 13 C NMR (125 MHz) (delta, selected. . .

DETD . . . washed with brine and dried over magnesium sulfate. The solvent was then removed and the material flash chromatographed on silica **gel** eluting with 25% acetone in hexane. Yield 10 1 mg: MS (FAB) m/e: M+K=946.

DETD . . . was quenched by the addition of 10 mL of water. The tetrahydrofuran was removed, and the residue loaded onto silica **gel** and eluted with 25% acetone in hexane. Yield 0.60 g: MS (FAB) m/e: M+K=897, M+H=859.

DETD . . . mL) were stirred at 45.degree. C. for 60 hours. Solvent was removed in vacuo and the product purified on silica **gel** eluting with 10% ethanol/dichloromethane. Title compound of Example 388a: Yield: 10.2 g; MS(FAB) m/z: M+K=859. Title compound of Example 388b:. . .

DETD . . . at 0.degree. C. After being stirred at room temperature overnight, the reaction mixture was poured over a column of silica **gel** (2 g) in ether and eluted with ether. The semi-pure product was purified by silica **gel** chromatography (10 g) eluting with 2% isopropanol/dichloromethane. Yield: 0.245 g; MS(FAB) m/z: M+NH.sub.4 =849.

DETD . . . at 0.degree. C. After being stirred at room temperature overnight, the reaction mixture is poured over a column of silica **gel** (2 g) in ether and eluted with ether. The semi-pure product is purified by silica **gel** chromatography (10 g) eluting with 2% isopropanol/dichloromethane.

DETD . . . added and stirred at room temperature for 24 hours. Solvent was removed in vacuo and the crude purified by silica **gel** (40 g) eluting with 40% acetone/hexanes. The product was further purified by silica **gel** (40 g) eluting with 3% isopropanol/dichloromethane. Yield: 0.3 g; MS(FAB) m/z: M+K=882.

DETD . . . mixture and stirred at room temperature for 24 hours. Solvent was removed in vacuo and the product purified on silica **gel** with 30% acetone/hexanes elution. Yield: 0.7 g; MS(FAB) m/z: M+H=888.

DETD . . . mixture and stirred at room temperature for 24 hours. Solvent is removed in vacuo and the product purified on silica **gel** with 30% acetone/hexanes elution.

DETD . . . once with saturated brine, dried over magnesium sulfate and solvent removed in vacuo. The solid residue was purified by silica **gel** (200 g) eluting with 25% acetone/hexanes. Yield: 8.5 g; MS (FAB) m/e: M+K=995.

DETD . . . g) in dichloromethane (1 mL) and stirred at room temperature for 12 hours. The reaction mixture was purified by silica **gel** chromatography (25 g) eluting with 25% acetone/hexanes. Yield: 0.4 g; MS (FAB) m/e: M+K=943.

DETD . . . g) in dichloromethane (1 mL) and stirred at room temperature for 12 hours. The reaction mixture was purified by silica **gel** chromatography (25 g) eluting with 60% acetone/hexanes. Yield: 0.25 g; MS (FAB) m/e: M+K=986.

DETD . . . washed once with brine, dried over magnesium sulfate and solvent removed in vacuo. The solid residue was purified by silica **gel** (20 g) eluted with 30% acetone/hexanes. Yield: 1.1 g.

DETD . . . acid. The organic phase is dried over magnesium sulfate and solvent removed in vacuo. The product is purified by silica **gel** chromatography eluting with 40% acetone in hexanes.

DETD . . . mL) was stirred at room temperature for 1 hour. Ethanol was removed in vacuo and the crude purified by silica **gel** chromatography (30 g) eluting with 20% acetone in hexanes. Yield: 0.34 g. MS (FAB) m/e: M+K=933.

DETD . . . mL) was stirred at room temperature for 1 hour. Ethanol was removed in vacuo and the crude purified by silica **gel** chromatography (30 g) eluting with 20% acetone in hexanes. Yield: 0.45 g. MS (FAB) m/e: M+K=857.

DETD . . . mL) was stirred at room temperature for 1 hour. Ethanol was removed in vacuo and the crude purified by silica **gel** chromatography (30 g) eluting with 20% acetone in hexanes. Yield: 0.33 g. MS (FAB) m/e: M+K=885.

DETD . . . added, and the reaction was then stirred at room temperature for three days. The reaction mixture was passed through silica **gel**, using 10-25% acetone in hexane as an elutant to obtain semi-pure title compound (1.25 g) in 65% yield. MS (FAB). . .

DETD . . . chloride were used and stirred at room temperature for three days. 1.45 g of pure compound was isolated after silica **gel** column chromatography, followed by normal phase HPLC purification in 71% yield. MS (FAB) m/z: M+H=1007. M+K=1045. The obtained product (1.4. . .

DETD . . . chloride were used and stirred at room temperature for three days. 2.0 g of semi-pure compound was isolated after silica **gel** column chromatography, followed by normal phase HPLC purification. MS (FAB) m/z: M+H=1090. The obtained product (2.0 g, 1.90 mmol) was. . .

DETD . . . used and stirred at room temperature for one over night and at 40.degree. C. for an additional day. After silica **gel** column chromatography eluting with 10%-acetone in hexane, followed by normal phase HPLC purification using 40% acetone in hexane as an. . .

DETD . . . chloride were used and stirred at room temperature for three days. 1.59 g of semi-pure compound was isolated after silica **gel** column chromatography, initially eluting with 10%-acetone in hexane, followed by 10% methanol in methylene chloride in 82% yield. MS (FAB). . .

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AB The present invention provides purified and isolated polynucleotides encoding Type I ribosome-inactivating proteins (RIPs) such as gelonin and analogs of the RIPs having a cysteine available for disulfide bonding to targeting molecules. Vectors comprising the polynucleotides and host cells transformed with the vectors are also provided. The RIPs and RIP analogs are particularly suited for use as components of cytotoxic therapeutic agents of the invention which include gene fusion products and immunoconjugates. Cytotoxic therapeutic agents or immunotoxins according to the present invention may be used to selectively eliminate any cell type to which the RIP component is targeted by the specific binding capacity of the second component of the agent, and are suited for treatment of diseases where the elimination of a particular cell type is a goal, such as autoimmune disease, cancer and graft-versus-host disease.

AN 95:43358 USPATFULL
TI Materials comprising and methods of preparation and use for ribosome-inactivating proteins
IN Bernhard, Susan L., Menlo Park, CA, United States
Better, Marc D., Los Angeles, CA, United States
Carroll, Steve F., Walnut Creek, CA, United States
Lane, Julie A., Castro Valley, CA, United States
Lei, Shau-Ping, Los Angeles, CA, United States
PA XOMA Corporation, Berkeley, CA, United States (U.S. corporation)
PI US 5416202 19950516 <--
AI US 1992-988430 19921209 (7)
RLI Continuation-in-part of Ser. No. US 1992-901707, filed on 19 Jun 1992 which is a continuation-in-part of Ser. No. US 1991-787567, filed on 4 Nov 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Zitomer, Stephanie W.
LREP Marshall, O'Toole, Gerstein, Murray & Borun
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 9 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 3527
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5416202 19950516 <--
DETD . . . of autoimmune diseases are systemic lupus erythematosus, scleroderma diseases (including lichen sclerosus, morphea and lichen planus), rheumatoid arthritis, chronic thyroiditis, **pemphigus vulgaris**, diabetes mellitus type 1, progressive systemic sclerosis, aplastic anemia, myasthenia gravis, myositis, Sjogrens disease, Crohn's disease, ulcerative colitis, and primary. . .
DETD . . . reactions of a host. Preferred immunosuppressive agents include
prednisone, prednisolone, DECADRON (Merck, Sharp & Dohme, West Point, Pa.), cyclophosphamide, cyclosporine, 6-**mercaptopurine**, methotrexate, **azathioprine** and i.v. gamma globulin or their combination. Preferred potentiators include monensin, ammonium chloride,
perhexiline, verapamil, amantadine and chloroquine. All of. . .
DETD Anti-T cell immunotoxins may be formulated into either an injectable or **topical** preparation. Parenteral formulations are known and are suitable for use in the invention, preferably for intramuscular or intravenous administration. The. . .
DETD Anti-T cell immunotoxin is formulated into **topical** preparations for local therapy by including a therapeutically effective concentration of anti-T cell immunotoxin in a dermatological vehicle. The amount of anti-T cell immunotoxin to be administered, and the anti-T
cell immunotoxin concentration in the **topical** formulations, depends upon the vehicle selected, the clinical condition of the patient, the systemic toxicity and the stability of the. . . depending upon clinical experience with the patient in question or with similar patents. The concentration of anti-T cell immunotoxin for **topical** formulations is in the range of greater than from about 0.1 mg/ml to about 25 mg/ml. Typically, the concentration of anti-T cell
immunotoxin for **topical** formulations is in the range of greater than from about 1 mg/ml to about 20 mg/ml. Solid dispersions of anti-T. . . vehicle may be useful with 1% w/w hydrogel vehicles in the treatment of skin inflammation. Suitable vehicles, in addition to **gels**, are oil-in-water or water-in-oil emulsions using mineral

oils, petroleum and the like.

DETD . . . by the use of a transdermal therapeutic system [Barry, Dermatological Formulations, p. 181 (1983) and literature cited therein]. While such **topical** delivery systems have been designed for transdermal administration of low molecular weight drugs, they are capable of percutaneous delivery. They. . .

DETD **Topical** preparations of anti-T cell immunotoxin either for systemic or local delivery may be employed and may contain excipients as described above for parenteral administration and other excipients used in a **topical** preparation such as cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Any pharmacologically-acceptable buffer may be used, e.g., Tris or phosphate buffers. The **topical** formulations may also optionally include one or more agents variously termed enhancers, surfactants, accelerants, adsorption promoters or penetration enhancers, such. . . pharmacological inertness, non-promotive of body fluid or electrolyte loss, compatible with anti-T cell immunotoxin (non-inactivating), and capable of formulation into **creams**, **gels** or other **topical** delivery systems as desired.

DETD . . . cell immunotoxin may also be administered via microspheres, liposomes or other microparticulate delivery systems placed in certain tissues including blood. **Topical** preparations are applied daily directly to the skin or mucosa and are then preferably occluded, i.e., protected by overlaying a bandage, polyolefin film or other barrier impermeable to the **topical** preparation.

DETD . . . .degree. C. for 16 hours. Next, the RNA was pelleted by centrifugation for 20 minutes at 4 .degree. C. The **pellet** was washed with 5 ml of 2M LiC1, recentrifuged and resuspended in 2 ml of water. The RNA was precipitated. . .

DETD . . . a gelonin gene fragment. When products of the expected DNA size were identified as ethidium bromide-stained DNA bands on agarose **gels**, the DNA was treated with T4 DNA polymerase and then purified from an agarose **gel**. Only the primer pair consisting of primers designated gelo-7 and gelo-5 yielded a relatively pure product of the expected size.. . .

DETD . . . XhoI and EcoRI, and the resulting 208 bp fragment encoding amino acids 185-251 of gelonin was purified from an agarose **gel** . This fragment was ligated adjacent to the NcoI to EcoRI fragment from pING3823 encoding amino acids 37-185 of gelonin to. . .

DETD . . . the ligated DNA was amplified by PCR with oligonucleotides Gelo-9 (SEQ ID NO: 20) and Gelo-16. The sequence of primer **Gel** -16 is set out below.

DETD The PCR product was size-fractionated on an agarose **gel** and DNAs larger than 300 bp were cloned into SmaI cut pUC18. Several clones were sequenced with the primer Gelo-18,. . .

DETD . . . treated with T4 polymerase and cut with NcoI. The resulting 100 bp 5'-end DNA fragment was isolated from an agarose **gel** and ligated adjacent to the 120 bp pelB leader fragment from plC100 (cut with SstI, treated with T4 polymerase and. . .

DETD . . . lysine.sub.10, asparagine.sub.60, isoleucine103, aspartic acid.sub.146, arginine.sub.184, serine.sub.215, asparagine.sub.239, lysine.sub.244, aspartic acid.sub.247, and lysine.sub.248, and the analogs have respectively been designated **Gel**.sub.c10, **Gel**.sub.c60, **Gel**.sub.c103, **Gel**.sub.c146, **Gel**.sub.c184, **Gel**.sub.c215, **Gel**.sub.c239,

Gel.sub.c244, **Gel**.sub.c247, and **Gel**.sub.c248.

DETD . . . a non-cysteine residue. Specifically, the cysteine at position 50 was replaced with an alanine residue, creating a gelonin analog (designated **Gel**.sub.c44) which has a cysteine available for disulfide bonding at position 44. Conversely, the cysteine at position 44 was replaced with an alanine residue, resulting in an analog (designated **Gel**.sub.c50) which has a cysteine available for disulfide bonding at position 50. The combined series of the foregoing twelve analogs thus. . .

DETD Another gelonin analog (**Gel**.sub.c44AC50A) was constructed in which both native gelonin cysteines were replaced with alanines. Two additional analogs were constructed that have alanine. . .

DETD . . . with EcoRI and XhoI, purified, and was inserted into plasmid pING3825 in a three-piece ligation. The DNA sequence of the **Gel**.sub.c247 variant was then verified. The plasmid containing the sequence encoding **Gel**.sub.c247 was designated pING3737 (deposited on Jun. 9, 1992 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md 20852. . .

DETD . . . the amino acid at position 248 (a lysine) of gelonin with the mutagenic oligonucleotides GeloC-1 and GeloC-2 to generate analog **Gel**.sub.c248 in plasmid pING3741, and a cysteine residue was introduced at amino acid position 239 (a lysine) with primers GeloC-9 and GeloC-10 to generate analog **Gel**.sub.239 in plasmid pING3744.

DETD . . . residue was introduced at amino acid 244 (a lysine) of gelonin with mutagenic primers GeloC-5 and GeloC-6 to generate analog **Gel**.sub.c244 in the plasmid designated pING3736. This variant was prepared by PCR using plasmid pING3734 as template DNA rather than pING3825.. . .

DETD . . . and Gelo-11. The PCR product was cut with PstI and NcoI, purified, and cloned back into pING3825 to generate analog **Gel**.sub.c10 in the plasmid designated pING3746 (deposited on Jun. 9, 1992 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville,. . .

DETD . . . mutagenic oligos, GeloC-15 and GeloC-16, in conjunction with oligos ara B2 and Gelo-11 in the same manner as for the **Gel**.sub.c10 variant. The plasmid encoding the **Gel**.sub.c60 analog was designated pING3749.

DETD . . . at residue 103 also introduced an AflIII restriction site which was verified in the cloned gene. The plasmid containing the **Gel**.sub.c103 analog was designated pING3760.

DETD . . . also introduced an NsiI restriction site which was verified in the cloned gene. The plasmid containing the sequence encoding the **Gel**.sub.c184 variant was designated pING3761.

DETD . . . and BglII, and cloned back into the vector portion of pING3825 to generate pING3747 (ATCC 69101). This analog was designated **Gel**.sub.c44 because it contains a cysteine available for disulfide bonding at amino acid position 44.

DETD . . . DNA was cut with NcoI and BglII, and cloned into a gelonin vector, generating pING3756. The variant generated was designated **Gel**.sub.c50.

DETD . . . has no cysteine residues available for conjugation. The plasmid encoding the analog was designated pING3750. The analog generated was designated **Gel**.sub.c44AC50A.

DETD . . . the gelonin analog. Plasmid pING3824 was cut with NcoI and XhoI

and the vector fragment was purified in an agarose **gel**.
pING3750 was cut with NcoI and EcoRI and pING3737 was cut with EcoRI and
XhoI. The NcoI-EcoRI fragment encodes the. . .
DETD . . . while pING3750 was cut with NcoI and XhoI. Each of the insert fragments were purified by electrophoresis in an agarose **gel**, and the fragments were ligated into a PstI and XhoI digested vector fragment. The resulting vector was designated pING3753.
DETD . . . its analogs exhibit activity in the RLA comparable to that of native gelonin. For some of the analogs (such as **Gel** .sub.c239), RLA activity was diminished.
DETD TABLE 1

Toxin	IC.sub.50 (pM)
RTA 30	2.5
Gelonin	15
rGelonin	11
Gel.sub.C10	60
Gel.sub.C44	20
Gel.sub.C50	47
Gel.sub.C60	26
Gel.sub.C239	955
Gel.sub.C244	32
Gel.sub.C247	12
Gel.sub.C248	47
Gel.sub.C44AC50A	16
GelC10.sub.C44AC50A	7
GelC247.sub.C44AC50A	20

DETD Specifically, the **Gel**.sub.c248 analog (3.8 mg/ml) was treated with 2 mM DTT for 60 minutes in 0.1 M NaPhosphate, 0.25 M NaCl, pH 7.5 buffer. The **Gel**.sub.c244 variant (7.6 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 M NaPhosphate, 0.25 M NaCl, pH 7.5 buffer. The **Gel**.sub.c247 analog (4 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 M NaPhosphate, 0.5 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. The **Gel**.sub.c239 variant (3.2 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 m NaPhosphate, 0.5 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. The **Gel**.sub.c44 analog (4.2 mg/ml) was treated with 0.1 mM DTT for 30 minutes in 0.1 M NaPhosphate, 0.1 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. Lastly, the **Gel** .sub.c10 variant (3.1 mg/ml) was treated with 1 mM DTT for 20 minutes in 0.1 M NaPhosphate, 0.1 M NaCl, pH. . .
DETD Specifically, for conjugation with **Gel**.sub.c248 and **Gel**.sub.c244, murine H65 antibody at 4 mg/mL was derivitized with 18x M2IT and 2.5 mM DTNB in 25 mM TEOA, 150. . .
DETD For conjugation with **Gel**.sub.c247 and **Gel**.sub.c239, H65 antibody at 4.7 mg/mL was derivitized with 20x M2IT and 2.5 mM DTNB in 25 mM TEOA 150 mM. . .
DETD Before reaction with **Gel**.sub.c44, H65 antibody at 5.8 mg/mL was derivitized with 20x m2IT and 2.5 mM DTNB in 25 mM TEOA, 150 mM. . .
DETD For conjugation with **Gel**.sub.c10, H65 antibody at 2.2 mg/mL was derivitized with 10x m2IT and 2.5 mM DTNB in 25 mM TEOA, 150 mM. . .
DETD . . . set up for each analog: 23 mg (in 7.2 ml) of H65-M2IT-TNB were

mixed with a 5-fold molar excess of **Gel**.sub.C248 (23 mg in 6 ml) for 2 hours at room temperature, then for 18 hours overnight at 4.degree. C.; 23 mg (in 7.3 ml) of H65-m2IT-TNB were mixed with a 5-fold

molar excess of **Gel**.sub.C244 (23 mg in 3 ml) for 3 hours at room temperature, then for 18 hours overnight at 4.degree. C.; 9 mg (in 2.8 mL) of H65-m2IT-TNB were mixed with a 5-fold molar excess of **Gel**.sub.C247 (9 mg in 2.25 mL) for 2 hours at room temperature, then for 5 nights at 4.degree. C.; 9 mg (in 2.8 mL) of H65-m2IT-TNB were

mixed with a 5-fold molar excess of **Gel**.sub.C239 (9mg in 2.6 mL) for 2 hours at room temperature, then at 4.degree. C. for 3 days; 12

mg (in 1.9 mL) of H65-m2IT-TNB were mixed with a 5.6-fold molar excess of **Gel**.sub.C44 (13.44 mg in 3.2 mL) for 4.5 hours at room temperature, then 4.degree. C. overnight; and 11 mg of H65-m2IT-TNB were

mixed with a 5-fold molar excess of **Gel**.sub.C10 (11 mg in 3.5 mL) for 4 hours at room temperature, then at 4.degree. C. overnight.

DETD . . . 1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction solution was then loaded onto a **gel** filtration column [Sephadex G-150 (Pharmacia) in the case of **Gel**.sub.C248, GelC.sub.247, **Gel**.sub.C244 and **Gel**.sub.C239 and an AcA-44 column (IBF Biotecnics, France) in the case of **Gel**.sub.C44 and **Gel**.sub.C10]. The reactions were run over the **gel** filtration columns and eluted with 10 mM Tris, 0.15M NaCl pH 7. The first peak off each column was loaded. . .

DETD . . . the number of toxins per antibody (T/A ratio). The yield of final product for each analog conjugate was as follows: **Gel**.sub.C248, 17 mg with a T/A ration of 1.6; **Gel**.sub.C247, 1.1 mg with a T/A ratio of 1; **Gel**.sub.C244, 4.5 mgs with a T/A ratio of 1.46; **Gel**.sub.C239, 2.9 mg with a T/A ratio of 2.4; **Gel**.sub.C44, 7.3 mg with a T/A ratio of 1.22; and **Gel**.sub.C10, 6.2 mg with a T/A ratio of 1.37. Conjugation efficiency (i.e.,

conversion of free antibody to immunoconjugate) was significantly greater (.about.80%) for some analogs (**Gel**.sub.C10, **Gel**.sub.C44, **Gel**.sub.C239, **Gel**.sub.C247, and **Gel**.sub.C248) than for others (.about.10%, **Gel**.sub.C244).

DETD Analogs **Gel**.sub.C247 and **Gel**.sub.C44 were conjugated to various chimeric [cFab, cFab' and cF(ab').sub.2] and "human engineered"[he1 Fab, he2 Fab, he3 Fab, he1 Fab'. . .

DETD The chimetic H65 antibody fragments were conjugated to **Gel**.sub.C247 analog basically as described below for conjugation of human engineered Fab and Fab' fragments to **Gel**.sub.C247 and **Gel**.sub.C44.

DETD (a) he1 Fab-**Gel**.sub.C247

DETD . . . of 2.5 mM DTNB. The reaction was allowed to proceed for 30 minutes at room temperature, then desalted on GF05 (**gel** filtration resin) and equilibrated in 0.1 M Na Phosphate, 0.2M NaCl, pH 7.5. A linker number of 1.8 linkers per. . . Fab was calculated based

on the DTNB assay. The he1 Fab-M2IT-TNB was concentrated to 3.7 mg/mL prior to conjugation with **Gel**.sub.C247.

DETD **Gel**.sub.C247 at 12.8 mg/mL in 10 mM Na Phosphate, 0.3M NaCl, was treated with 1 mM DTT, 0.5 mM EDTA for. . . Phosphate, 0.2M NaCl,

pH 7.5. Free thiol content was determined to be 0.74 moles of :free SH

per mole of **Gel**.sub.C247 using the DTNB assay. The gelonin was concentrated to 8.3 mg/mL prior to conjugation with activated antibody.

DETD The conjugation reaction between the free thiol on **Gel** .sub.C247 and the derivitized he1 Fab-M2IT-TNB, conditions were as follows. A 5-fold excess of the gelonin analog was added to activated. . . with 1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction solution was loaded onto a **gel** filtration column (G-75) equilibrated with 10 mM Tris, 150 mM NaCl, pH 7. The first peak off this column was. . .

DETD (b) he1 Fab '-**Gel**.sub.C247

DETD . . . mM DTNB. The reaction was allowed to proceed for 1 hour at room

temperature then it was desalted on GF05 (**gel** filtration resin) and equilibrated in 0.1 M Na Phosphate, 0.2M NaCl, pH 7.5. A linker number of 1.6 linkers per. . . Fab' was calculated based on the DTNB assay. The he1 Fab'-M2IT-TNB was concentrated to 3.7 mg/mL prior to conjugation with **Gel**.sub.C247

DETD The **Gel**.sub.C247 at 77 mg/mL was diluted with in 10 mM Na Phosphate, 0.1M NaCl to a concentration of 5 mg/mL, treated. . . Phosphate, 0.2M NaCl, pH 7.5. Free thiol content was determined to be 1.48 moles of free SH per mole of **Gel**.sub.C247 using the DTNB assay. The **Gel**.sub.C247 was concentrated to 10 mg/mL prior to conjugation with activated he1 Fab'-M2IT-TNB.

DETD For the reaction between the free thiol on **Gel**.sub.C247 and the derivitized he1 Fab'-M2IT-TNB, conditions were as follows. A 5.7-fold molar excess of gelonin was added to activated he1. . . with

1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction solution was loaded onto a **gel** filtration column (AcA54) equilibrated with 10 mM Tris, 250 mM NaCI, pH 7.5. The first peak off this column was. . .

DETD (c) he2 Fab **Gel**.sub.C44

DETD . . . DTNB. The reaction was allowed to proceed for 20 minutes at room temperature and was then alesalted on a GF05-LS (**gel** filtration) column, equilibrated in 0.1M Na Phosphate, 0.2M NaCl with 0.02% Na azide. A linker number of 1.7 linkers per Fab-M2IT-TNB was calculated based on the DTNB assay. After derivitization and **gel** filtration, the he2 Fab concentration was 5.2 mg/mL.

DETD **Gel**.sub.C44 at 8.33 mg/mL in 10 mM Na Phosphate, pH 7.2 was treated with 5 mM DTT and 0.5 mM EDTA. . . % Na azide, pH 7.5. Free thiol content was determined to be 0.83 moles of free SH per mole of **Gel**.sub.C44 using the DTNB assay. The gelonin was concentrated to 11.4 mg/mL prior to conjugation with activated he2 Fab.

DETD The conjugation reaction conditions between the free thiol on **Gel**.sub.C44 and the derivitized he2 Fab-M2IT-TNB were as follows. A 3-fold excess of the gelonin analog was added to activated he2. . .

DETD . . . incubation with 1:1 mole cysteamine to linker for 15 minutes at

room temperature. The quenched reaction as loaded onto a **gel** filtration column (G-75) equilibrated in 10 mM Tris, 0.15M NaCl, pH 7. The first peak off this column was diluted. . .

DETD (d) he3 Fab **Gel**.sub.C44

DETD . . . DTNB. The reaction was allowed to proceed for 45 minutes at room temperature and was then alesalted on a GF05-LS (**gel** filtration) column, equilibrated in 0.1M Na Phosphate, 0.2M NaCl with 0.02% Na azide. A linker number of 1M2IT per Fab-M2IT-TNB was calculated

based on the DTNB assay. After derivitization and **gel** filtration, the he3 Fab concentration was 5.3 mg/mL.

DETD **Gel**.sub.C44 at 7.8 mg/mL in 0.1M Na Phosphate, 0.1M NaCl, pH 7.5 was treated with 1.5 mM DTT and 1 mM. . . 0.02% Na azide, pH 7.5.

Free thiol content was determined to be 0.66 moles of free SH per mole of **Gel**.sub.C44 using the DTNB assay. The gelonin was concentrated to 5.2 mg/mL prior to conjugation with activated he3 Fab.

DETD The conjugation reaction conditions between. the free thiol on **Gel**.sub.C44 and the derivitized he3 Fab-M2IT-TNB were as follows. A 5-fold excess of the gelonin analog was added to activated he3. . .

DETD TABLE 2

IC.sub.50 (PM T) Conjugate	HSB2 Cells	PBMCs
H65-RTA	143	459
H65-(M2IT)-S--S-(M2IT)-Gelonin	1770	81
H65-(M2IT)-S--S-(M2IT)-rGelonin	276	75
H65-(M2IT)-S--S-**Gel**.sub.C10	140	28
H65-(M2IT)-S--S-**Gel**.sub.C44	99	51
H65-(M2IT)-S--S-**Gel**.sub.C239	2328	180
H65-(M2IT)-S--S-**Gel**.sub.C244	>5000	>2700
H65-(M2IT)-S--S-**Gel**.sub.C247	41	35
H65-(M2IT)-S--S-**Gel**.sub.C248	440	203
cH65-RTA.sub.30	60	400
cH65-(M21T)-S--S-(M2IT)-Gelonin	1770	140
cH65-(M2IT)-S--S-(M2IT)-rGelonin	153	120
cH65-(M2IT-S--S-**Gel**.sub.C239	>7000	290
cH65-(M2IT-S--S-**Gel**.sub.C247	34	60
cH65-(M21T)-S--S-**Gel**.sub.C248	238	860

DETD . . . conjugates were at least as active as native and recombinant gelonin conjugates. Importantly, however, some of the conjugates (for example, **Gel**.sub.C10, **Gel**.sub.C44 and **Gel** .sub.C247) exhibited an enhanced potency against PBMCs compared to native and recombinant gelonin conjugates, and also exhibited an enhanced level of. . .

DETD TABLE 3

IC.sub.50 (pM T) Conjugate	HSB2 Cells	PBMCs
cFab'-RTA 30	530	1800
cFab'-rGelonin	135	160
cFab'-**Gel**.sub.C247		

	48	64
cF(ab').sub.2 -RTA 30		
	33	57
cF(ab').sub.2 -rGelonin		
	55	34
cF(ab').sub.2 **Gel**.sub.C247		
	23	20
cF(ab').sub.2 -**Gel**.sub.C248		
	181	95

DETD TABLE 4

IC.sub.50 (pM T)

Conjugate	HSB2 Cells	Extent of Kill
hel Fab'-**Gel**.sub.C247		
	57.7	93%
hel Fab-**Gel**.sub.C247		
	180	94%
he2 Fab-**Gel**.sub.C44		
	363	91%
he3 Fab-**Gel**.sub.C44		
	191	93%
cFab'-**Gel**.sub.C247		
	47.5	93%
cF(ab').sub.2 -rGelonin		
	45.4	85%
F(ab').sub.2 -**Gel**.sub.C247		
	77.5	83%
cF(ab').sub.2 -**Gel**.sub.C247		
	23.2	85%

DETD The cFab '-**Gel**.sub.247 immunoconjugate is clearly more cytotoxic than cFab' conjugates with recombinant gelonin or RTA 30.

DETD TABLE 5

Conjugate	RC.sub.50 (mM)
H65-RTA 30	3.2
H65-(M2IT)-S--S-(M2IT)-gelonin	11.1
H65-(M2IT)-S--S-(M2IT)-rGelonin	3.0
H65-(M2IT)-S--S-**Gel**.sub.C10	2.5
H65-(M2IT)-S--S-**Gel**.sub.C44	0.6
H65-(M2IT)-S--S-**Gel**.sub.C239	774.0
H65-(M2IT)-S--S-**Gel**.sub.C244	1.2
H65-(M2IT)-S--S-**Gel**.sub.C247	0. 1
H65-(M2IT)-S--S-**Gel**.sub.C248	0.4
cH65-RTA 30	2.50
cH65-(M2IT)-S--S-(M2IT)-rGelonin	2.39
cH65-(M2IT)-S--S-**Gel**.sub.C247	

0.11
cH65-(M2IT)-S--S-**Gel**.sub.C248
0.32

DETD . . . that the stability of the bonds between the different gelonin proteins and H65 antibody varied greatly. With the exception of **Gel**.sub.C10 and **Gel**.sub.C239, most of the gelonin analogs resulted in conjugates with linkages that were somewhat less stable in this in vitro assay than the dual-linker chemical conjugate. The stability of the **Gel**.sub.C239 analog, however, was particularly enhanced.

DETD TABLE 6

Conjugate	RC.sub.50 (mM)
hel Fab'-**Gel**.sub.C247	0.07
cFab'-Gelonin	1.27
cFab'-**Gel**.sub.C247	0.08
cF(ab').sub.2 -RTA 30	1.74
cF(ab').sub.2 -rGelonin	2.30
cF(ab').sub.2 -**Gel**.sub.C247	0.09
cF(ab').sub.2 -**Gel**.sub.C248	0.32
he2 Fab-**Gel**.sub.C44	0.46
he3 Fab-**Gel**.sub.C44	0.58

DETD The pharmacokinetics of gelonin analogs **Gel**.sub.C247, **Gel**.sub.C44 and **Gel**.sub.C10 linked to whole H65 antibody was investigated in rats. An IV bolus of 0.1 mg/kg of .sup.125 I-labelled immunoconjugate H65-(M2IT)-S-S-**Gel**.sub.C247, H65-(M2IT)-S-S-**Gel**.sub.C44 or H65-(M2IT)-S-S-**Gel** .sub.C10 was administered to male Sprague-Dawley rats weighing 134-148 grams. Serum samples were collected from the rats at 3, 15, 30. . .

DETD . . . 7

IC	Vc (ml/kg)	Cl (ml/hr/kg)	MRT (hours)	Alpha (hours)	Beta (hours)
H65	65.3 .+-. 11.0	.+-. 16.5	.+-. 2.3	.+-. 20.5	.+-.
Gel.sub.C247 n = 32	3.4	0.4	1.9	0.2	3.0
H65 **Gel**.sub.C44	61.9 .+-. 4.1	.+-. 22.7	.+-. 3.0	.+-. 17.8	.+-.

n = 38 2.4 0.1 0.7 0.7 0.8
H65 **Gel**.sub.C10
59.2 .+-.
2.5 .+-. 42.7 .+-.
3.3 .+-.
32.9 .+-.
n = 45 1.3 0.04 1.1 0.3 1.1
p-value 0.176 <0.0001 <0.0001
. . . .
DETD The **Gel**.sub.C247 immunoconjugate was found to have .alpha. and .beta. half lives of 2.3 and 20 hours, with a total mean residence. . . 96 hour time points were excluded from analysis because of the poor resolution of immunoconjugate associated radioactivity on the SDS-PAGE **gel** for these serum samples.
DETD Because in vitro studies suggested that the **Gel**.sub.C10 immunoconjugate had greater disulfide bond stability, it was anticipated that its half lives in vivo would be longer relative to. . . of the immunoconjugate. The .beta. half life of the immunoconjugate was about 33 hours compared to 20 hours for the **Gel**.sub.C247 conjugate. The total mean residence time was also much greater for the **Gel** .sub.C10 immunoconjugate (42 hours versus 42 hours for the **Gel** .sub.247 conjugate). In addition, the clearance of the **Gel** .sub.C10 immunoconjugate was 2.5 ml/hr/kg, about four times less than that of the **Gel**.sub.C247 immunoconjugate (11 ml/hr/kg). As also predicted from the in vitro disulfide stability data, the clearance of the **Gel**.sub.C44 immunoconjugate was intermediate between those of the **Gel**.sub.C10 and **Gel**.sub.C247 immunoconjugates.
DETD Based on these studies, the **Gel**.sub.C10 analog conjugated to H65 antibody has greater in vivo stability than the **Gel** .sub.C44 and **Gel**.sub.C247 analogs conjugated to H65 antibody (as determined by the longer mean residence time and clearance rates), although the properties of the **Gel**.sub.C44 immunoconjugate more closely resembled those of the **Gel**.sub.C10 immunoconjugate than the **Gel**.sub.C247 immunoconjugate.
DETD The pharmacokinetics of **Gel**.sub.C247 and **Gel**.sub.C44 analogs linked to human engineered H65 Fab fragments were also investigated in rats. An IV bolus of 0.1 mg/kg of .sup.125 l-labelled he1 H65 Fab-**Gel**.sub.C247, he2 H65 Fab-Gelc44 or he3 H65 Fab-**Gel**.sub.C44 was administered to male Sprague-Dawley rats weighing 150-180 grams. Serum samples were collected at 3, 5, 15, 20, 30, and. . .
DETD TABLE 8

IC	Vc (ml/kg)	Vss (ml/hr/kg)	Cl (ml/hr/kg)	MRT (hours)	Alpha (hours)	Beta (hours)
he1 **Gel**.sub.C247	48 .+-. 3	133 .+-. 7	62 .+-. 3	2.1 .+-. 0.1	0.33 .+-. 0.03	

3.0 fixed

n = 27

he2 **Gel**.sub.C44

54 .+-. 5

141 .+-. 8

53 .+-. 3

2.7 .+-. 0.2

0.37 .+-. 0.04

3.1 fixed

n = 28

he3 **Gel**.sub.C44

77 .+-. 6

140 .+-. 20

57 .+-. 3

2.5 .+-. 0.4

0.58 .+-. 0.11

3.0 .+-. 1.0

n = 33

DETD Comparing the three immunoconjugates, the pharmacokinetics of he1 H65 Fab-**Gel**.sub.C247, he2 H65 Fab-**Gel**.sub.C44 and he3 Fab-**Gel**.sub.C44 were very similar, having similar alpha and beta half-lives, mean residence times, and clearance, particularly when comparing parameters obtained from the ELISA assayed curves. This is in contrast to their whole antibody immunoconjugate counterparts, where the clearance of **Gel**.sub.C247 immunoconjugate (11 ml/kg/hr) was three-fold greater than that of **Gel**.sub.C44 immunoconjugate (4 ml/kg/hr). This suggests that cleavage of the disulfide bond linking the Fab fragment and gelonin is not as. . .

DETD . . . severe combined immunodeficient mouse model was utilized to evaluate the in vivo efficacy of various immunoconjugates comprising the gelonin analogs **Gel**.sub.C247 and **Gel**.sub.C44. Immunoconjugates were tested for the capacity to deplete human blood cells expressing the CD5 antigen.

DETD . . . CD5 Plus (XOMA Corporation, Berkeley, Calif.) is mouse H65 antibody chemically linked to RTA and is a positive control. 0X19 Fab-**Gel**.sub.C247 is a negative control immunoconjugate. The 0.times.19 antibody (European Collection of Animal Cell Cultures #84112012) is a mouse anti-rat CD5. . .

DETD . . . Blood

CD5 Plus	+	+
cH65 F(ab').sub.2	-	-
cH65 Fab'	-	-
H65-rGEL	+	+
cH65 F(ab').sub.2 -rGel	+	+
cH65 Fab'-rGel	+	+
cH65 F(ab').sub.2 -**Gel**.sub.c247	+	NT
cH65 Fab'-**Gel**.sub.c247	+	+
he1H65 Fab'-**Gel**.sub.c247	+	NT
cH65 Fab'-**Gel**.sub.c44	+	+

OX19 Fab-**Gel**.sub.c247

DETD . . . a portion of the Fd constant domain and the entire SLT gene segment was purified by electrophoresis on an agarose **gel**. pING3731 was digested with SinI and XhoI and the 760 bp gelonin gene was similarly purified. Plasmid pING4000 was digested. . .

DETD TABLE 13

IC.sub.50 (pMT) Fusion Protein	HSB2 Cells	CEM Cells
he3Fab-**Gel**.sub.C44 Gelonin:SLT::Fd (kappa)	165	173
Gelonin::RMA::Fd (kappa)	180	1007
	150	nt

DETD . . . T4 polymerase and then cut with XhoI. The resulting 728 bp fragment was then purified by electrophoresis on an agarose **gel**. This fragment was ligated into the vectors pING3755 and pING3748 (see Example 10), each digested with ScaI and XhoI. The. . .

DETD . . . cut with BamHI and the 760 bp fragment corresponding to amino acids 1-256 of BRIP was purified from an agarose **gel**. Concurrently, a unique XhoI site was introduced downstream of the 3'-end of the BRIP gene in pBS1 by PCR amplification. . .

DETD . . . and XhoI, and an 87 bp fragment containing the 3'-end of the BRIP gene was purified on a 5% acrylamide **gel**. The 760 and 87 bp purified BRIP fragments were ligated in the vector pING 1500 adjacent to the pelB leader. . .

DETD . . . was cut with PstI and XhoI, and the BRIP gene linked to the pelB leader was purified from an agarose **gel**. The expression vector pING3217, containing the arab promoter, was cut with PstI and XhoI and ligated to the BRIP gene.. . .

DETD . . . of BRIP with the altered amino acid was excised from pMB2X and the fragment was purified on a 5% acrylamide **gel**. This fragment along with an EcoRI to BamHI fragment containing the pelB leader sequence and sequences encoding the first 256. . .

DETD . . . BRIP analog, was treated with T4 polymerase, cut with XhoI, and the resulting fragment was purified on a 5% acrylamide **gel**. Concurrently, plasmid pING3322 was cut with BamHI, treated with T4 polymerase, cut with EcoRI, and the fragment containing the pelB. . .

DETD . . . and the 51 bp fragment, which encodes the carboxyl terminal portion of the analog, was purified on a 5% acrylamide **gel**. The fragment (corresponding to amino acids 268-276 of BRIP.sub.C270) was cloned in a three piece ligation along with the internal. . .

DETD . . . BRIP-(M2IT)-S-S-TNB was first reduced to BRIP-(M2IT)-SH by treatment with 0.5 mM DTT for 1 hour at 25.degree. C., desalted by **gel** filtration of Sephadex.RTM. GF-05LS to remove the reducing agent, and then mixed with antibody-(M2IT)-S-S-TNB.

DETD . . . 25.degree. C. to quenched any unreacted m2IT linkers on the antibody. The quenched reaction solution was promptly loaded onto a **gel** filtration column (AcA44) to remove unconjugated ribosome-inactivating protein. Purification was completed using soft

gel affinity chromatography on Blue Toyopearl.RTM. resin using a method similar to Knowles et al., Analyt. Biochem., 160, 440 (1987). Samples. . .

DETD The resulting 81 bp PCR product was purified on a 5% acrylamide **gel** and cloned into the SmaI site of pUC18. Three candidate clones were sequenced, and one clone, pMO110, was identified which. .

DETD . . . with momo-9 and momo-10, and the product was treated with T4 polymerase, cut with XhoI, and purified on an agarose **gel**. This gene fragment was ligated along with the 131 bp pelB leader fragment from pIC100 which has been generated by. . .

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AB The present invention provides purified and isolated polynucleotides encoding Type I ribosome-inactivating proteins and analogs thereof having a cysteine available for disulfide bonding to targeting molecules. Vectors comprising the polynucleotides and host cells transformed with the vectors are also provided. Preferred analogs according to the present invention are analogs of Type I ribosome-inactivating proteins (1) having a cysteine available for intermolecular disulfide bonding located at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and corresponding to a position on the surface of ricin A-chain in its natural conformation and (2) retaining ribosome-inactivating activity of the Type I ribosome-inactivating protein. The RIP analogs are particularly suited for use as components of cytotoxic therapeutic agents and, more specifically, as components of immunotoxins. Cytotoxic agents according to the present invention may be used to selectively eliminate any cell type to which the RIP component is targeted by the specific binding capacity of the second component, and are suited for treatment of diseases where the elimination of a particular cell type is a goal, such as autoimmune disease, cancer and graft-versus-host disease.

AN 94:112910 USPATFULL

TI Analogs of ribosome-inactivating proteins

IN Bernhard, Susan L., Menlo Park, CA, United States
Better, Marc D., Los Angeles, CA, United States
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PA Xoma Corporation, Berkeley, CA, United States (U.S. corporation)

PI US 5376546 19941227 <--

AI US 1992-901707 19920619 (7)

RLI Continuation-in-part of Ser. No. US 1991-787567, filed on 4 Nov 1991, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Zitomer, Stephanie W.

LREP Marshall, O'Toole, Gerstein, Murray & Borun

CLMN Number of Claims: 11

ECL Exemplary Claim: 1

DRWN 12 Drawing Figure(s); 21 Drawing Page(s)

LN.CNT 2422

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5376546 19941227 <--

DETD . . . of autoimmune diseases are systemic lupus erythematosus, scleroderma diseases (including lichen sclerosus, morphea and lichen

planus), rheumatoid arthritis, chronic thyroiditis, **pemphigus vulgaris**, diabetes mellitus type 1, progressive systemic sclerosis, aplastic anemia, myasthenia gravis, myositis, Sjogrens disease, Crohn's disease, ulcerative colitis, and primary. . .

DETD . . . reactions of a host. Preferred immunosuppressive agents include

prednisone, prednisolone, DECADRON (Merck, Sharp & Dohme, West Point, Pa.), cyclophosphamide, cyclosporine, 6-**mercaptopurine**, methotrexate, **azathioprine** and i.v. gamma globulin or their combination. Preferred potentiators include monensin, ammonium chloride,

perhexiline, verapamil, amantadine and chloroquine. All of. . .

DETD Anti-T cell immunotoxins may be formulated into either an injectable or **topical** preparation. Parenteral formulations are known and are suitable for use in the invention, preferably for intramuscular or intravenous administration. The. . .

DETD Anti-T cell immunotoxin is formulated into **topical** preparations for local therapy by including a therapeutically effective concentration of anti-T cell immunotoxin in a dermatological vehicle. The amount of anti-T cell immunotoxin to be administered, and the anti-T

cell immunotoxin concentration in the **topical** formulations, depends upon the vehicle selected, the clinical condition of the patient, the systemic toxicity and the stability of the. . . depending upon clinical experience with the patient in question or with similar patents. The concentration of anti-T cell immunotoxin for **topical** formulations is in the range of greater than from about 0.1 mg/ml to about 25 mg/ml. Typically, the concentration of anti-T cell

immunotoxin for **topical** formulations is in the range of greater than from about 1 mg/ml to about 20 mg/ml. Solid dispersions of anti-T. . . vehicle may be useful with 1% w/w hydrogel vehicles in the treatment of skin inflammation. Suitable vehicles, in addition to **gels**, are oil-in-water or water-in-oil emulsions using mineral oils, petroleum and the like.

DETD . . . by the use of a transdermal therapeutic system [Barry, Dermatological Formulations, p. 181 (1983) and literature cited therein]. While such **topical** delivery systems have been designed for transdermal administration of low molecular weight drugs, they are capable of percutaneous delivery. They. . .

DETD **Topical** preparations of anti-T cell immunotoxin either for systemic or local delivery may be employed and may contain excipients as

described above for parenteral administration and other excipients used in a **topical** preparation such as cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Any pharmacologically-acceptable buffer may be used, e.g.,

Tris or phosphate buffers. The **topical** formulations may also optionally include one or more agents variously termed enhancers, surfactants, accelerants, adsorption promoters or penetration enhancers,

such. . . pharmacological inertness, non-promotive of body fluid or electrolyte loss, compatible with anti-T cell immunotoxin (non-inactivating), and capable of formulation into **creams**, **gels** or other **topical** delivery systems as desired.

DETD . . . cell immunotoxin may also be administered via microspheres, liposomes or other microparticulate delivery systems placed in certain tissues including blood. **Topical** preparations are applied daily directly to the skin or mucosa and are then preferably occluded,

i.e., protected by overlaying a bandage, polyolefin film or other barrier impermeable to the **topical** preparation.

DETD . . . at 4.degree. C. for 16 hours. Next, the RNA was pelleted by centrifugation for 20 minutes at 4.degree. C. The **pellet** was washed with 5 ml of 2M LiCl, recentrifuged and resuspended in 2 ml of water. The RNA was precipitated. . .

DETD . . . a gelonin gene fragment. When products of the expected DNA size

were identified as ethidium bromide-stained DNA bands on agarose **gels**, the DNA was treated with T4 DNA polymerase and then purified from an agarose **gel**. Only the primer pair consisting of primers designated gelo-7 and gelo-5 yielded a relatively pure product of the expected size.. . .

DETD . . . XhoI and EcoRI, and the resulting 208 bp fragment encoding amino acids 185-251 of gelonin was purified from an agarose **gel** . This fragment was ligated adjacent to the NcoI to EcoRI fragment from pING3823 encoding amino acids 37-185 of gelonin to. . .

DETD . . . and Gelo-16. The sequence of primer Gelo-16 is set out below. ##STR8## The PCR product was size-fractionated on an agarose **gel** and DNAs larger than 300 bp were cloned into SmaI cut pUC18. Several clones were sequenced with the primer Gelo-18,. . .

DETD . . . treated with T4 polymerase and cut with NcoI. The resulting 100

bp 5'-end DNA fragment was isolated from an agarose **gel** and ligated adjacent to the 120 bp pelB leader fragment from plC100 (cut with SstI, treated with T4 polymerase and. . .

DETD . . . of one of the following residues: lysine.sub.10, asparagine.sub.60, asparagine.sub.239, lysine.sub.244, aspartate.sub.247, and lysine.sub.248, and the analogs have respectively

been designated **Gel**.sub.C10, **Gel**.sub.C60, **Gel**.sub.C230, **Gel**.sub.C244, **Gel**.sub.C247, and **Gel**.sub.C248,

DETD . . . a non-cysteine residue. Specifically, the cysteine at position 50 was replaced with an alanine residue, creating a gelonin analog (designated **Gel**.sub.C44) which has a cysteine available for disulfide bonding at position 44. The combined series of the foregoing seven analogs thus. . .

DETD . . . with EcoRI and XhoI, purified, and was inserted into plasmid pING3825 in a three-piece ligation. The DNA sequence of the **Gel** .sub.C247 variant was then verified. The plasmid containing the sequence

encoding **Gel**.sub.C247 was designated pING3737 (A.T.C.C. Accession No. 69009).

DETD . . . amino acid acid at position 248 (a lysine) of gelonin with the mutagenic oligonucleotides GeloC-1 and GeloC-2 to generate analog **Gel**.sub.C248 in plasmid pING3741, and a cysteine residue was introduced at amino acid position 239 (a lysine) with primers GeloC-9 and GeloC-10 to generate analog **Gel**.sub.C239 in plasmid pING3744.

DETD . . . residue was introduced at amino acid 244 (a lysine) of gelonin with mutagenic primers GeloC-5 and GeloC-6 to generate analog **Gel**.sub.C244 in the plasmid designated pING3736. This variant was prepared by PCR using plasmid pING3734 as template DNA rather than pING3825.. . .

DETD . . . and Gelo-11. The PCR product was cut with PstI and NcoI, purified, and cloned back into pING3825 to generate analog **Gel** .sub.10 in the plasmid designated pING3746 (A.T.C.C. Accession No. 69008).

DETD . . . two mutagenic oligos, GeloC-15 and GeloC-16, in conjunction

with oligos araB2 and Gelo-11 in the same manner as for the **Gel**.sub.C10 variant. The plasmid encoding the **Gel**.sub.C60 analog was designated pING3749.

DETD . . . with NcoI and BglII, and cloned back into the vector portion of pING3825 to generate pING3747. This analog was designated **Gel**.sub.44 because it contains a cysteine available for disulfide bonding at amino acid position 44.

DETD TABLE 1

Toxin	IC.sub.50 (pM)
RTA 30	2.5
Gelonin	15
rGelonin	11
Gel.sub.C10	60
Gel.sub.C44	20
Gel.sub.C239	955
Gel.sub.C244	32
Gel.sub.C247	12
Gel.sub.C248	47

DETD **Gel**.sub.C60 and the gelonin analog with both native cysteines replaced with alanines were both active in the RLA (data not shown).

DETD Specifically, the **Gel**.sub.C248 analog (3.8 mg/ml) was treated with 2 mM DTT for 60 minutes in 0.1 M NaPhosphate, 0.25 M NaCl, pH.7.5 buffer. The **Gel**.sub.C244 variant (7.6 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 M NaPhosphate, 0.25 M NaCl, pH 7.5 buffer. The **Gel**.sub.C247 analog (4 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 M NaPhosphate, 0.5 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. The **Gel**.sub.C239 variant (3.2 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 m NaPhosphate, 0.5 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. The **Gel**.sub.C44 analog (4.2 mg/ml) was treated with 0.1 mM DTT for 30 minutes in 0.1 M NaPhosphate, 0.1 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. Lastly, the **Gel**.sub.C10 variant (3.1 mg/ml) was treated with 1 mM DTT for 20 minutes in 0.1 M NaPhosphate, 0.1 M NaCl, pH. . .

DETD Specifically, for conjugation with **Gel**.sub.C248 and **Gel**.sub.C244, murine H65 antibody at 4 mg/mL was derivitized with 18x M2IT and 2.5 mM DTNB in 25 mM TEOA, 150. . .

DETD For conjugation with **Gel**.sub.C247 and **Gel**.sub.C239, H65 antibody at 4.7 mg/mL was derivitized with 20x M2IT and 2.5 mM DTNB in 25 mM TEOA 150 mM. . .

DETD Before reaction with **Gel**.sub.C44, H65 antibody at 5.8 mg/mL was derivitized with 20x m2IT and 2.5 mM DTNB in 25 mM TEOA, 150 mM. . .

DETD For conjugation with **Gel**.sub.C10, H65 antibody at 2.2 mg/mL was derivitized with 10x m2IT and 2.5 mM DTNB in 25 mM TEOA, 150 mM. . .

DETD . . . hours overnight at 4.degree. C.; 23 mg (in 7.3 ml) of H65-m2IT-TNB were mixed with a 5fold molar excess of **Gel**

.sub.C244 (23 mg in 3 ml) for 3 hours at room temperature, then for 18 hours overnight at 4.degree. C.; 9 mg (in 2.8 mL) of H65-m2IT-TNB were mixed with a 5-fold molar excess of **Gel**.sub.C247 (9 mg in 2.25 mL) for 2 hours at room temperature, then for 5 nights at 4.degree. C.;

9 mg. . . 4.degree. C. for 3 days; 12 mg (in 1.9 mL) of H65-m2IT-TNB were mixed with a 5.6-fold molar excess of **Gel**.sub.C44 (13.44 mg in 3.2 mL) for 4.5 hours at room temperature, then 4.degree. C. overnight; and 11 mg of H65-m2IT-TNB were mixed with a 5-fold molar excess of **Gel**.sub.C10 (11 mg in 3.5 mL) for 4 hours at room temperature, then at 4.degree. C. overnight.

DETD . . . 1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction solution was then loaded onto a **gel** filtration column [Sephadex G-150 (Pharmacia) in the case of **Gel**.sub.C248, **Gel**.sub.C247, **Gel**.sub.C244 and **Gel**.sub.239 and an AcA-44 column (IBF Biotecnics, France) in the case of **Gel**.sub.C44 and **Gel**.sub.C10]. The reactions were run over the **gel** filtration columns and eluted with 10 mM Tris, 0.15M NaCl pH 7. The first peak off each column was loaded. . .

DETD . . . the number of toxins per antibody (T/A ratio). The yield of final product for each analog conjugate was as follows: **Gel** .sub.C248, 17 mg with a T/A ration of 1.6; **Gel**.sub.C247, 1.1 mg with a T/A ratio of 1; **Gel**.sub.C244 , 4.5 mgs with a T/A ratio of 1.46; **Gel**.sub.C239, 2.9 mg with a T/A ratio of 2.4; **Gel**.sub.C44, 7.3 mg with a T/A ratio of 1.22; and **Gel** .sub.C10, 6.2 mg with a T/A ratio of 1.37. Conjugation efficiency (i.e., conversion of free antibody to immunoconjugate) was significantly greater (.about.80%) for some analogs (**Gel**.sub.C10, **Gel**.sub.C44, **Gel**.sub.C239, **Gel**.sub.C247, and **Gel**.sub.C248) than for others (.about.10%, **Gel** .sub.C244).

DETD Analog **Gel**.sub.C247 was conjugated to various chimeric [cFab, cFab' and cF(ab').sub.2] and "human engineered" [hel Fab, hel Fab' and hel F(ab').sub.2. . .

DETD The H65 antibody fragments were conjugated to **Gel**.sub.C247 analog basically as described below for conjugation of human engineered Fab and Fab' fragments to **Gel**.sub.C247.

DETD . . . of 2.5 mM DTNB. The reaction was allowed to proceed for 30 minutes at room temperature, then desalted on GF05 (**gel** filtration resin) and equilibrated in 0.1 M Na Phosphate, 0.2M NaCl, pH 7.5. A linker number of 1.8 linkers per. . . Fab was calculated based on the DTNB assay. The hel Fab-M2IT-TNB was concentrated to 3.7 mg/mL prior to conjugation with **Gel**.sub.C247.

DETD **Gel**.sub.C247 at 12.8 mg/mL in 10 mM Na Phosphate, 0.3M NaCl, was treated with 1 mM DTT, 0.5 mM EDTA for. . . 0.2 M NaCl, pH 7.5. Free thiol content was determined to be 0.74 moles of free SH per mole of **Gel**.sub.C247 using the DTNB assay. The gelonin was concentrated to 8.3 mg/mL prior to conjugation with activated antibody.

DETD . . . with 1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction solution was loaded onto a **gel** filtration column (G-75) equilibrated with 10 mM Tris, 150 mM NaCl, pH 7. The first peak off this column was. . .

DETD . . . mM DTNB. The reaction was allowed to proceed for 1 hour at room temperature then it was desalted on GF05 (**gel** filtration resin) and equilibrated in 0.1 M Na Phosphate, 0.2 M NaCl, pH 7.5. A linker number of 1.6 linkers. . . Fab' was calculated based on the DTNB assay. The hel Fab'-M2IT-TNB was concentrated to 3.7 mg/mL prior to

conjugation with **Gel**.sub.C247.

DETD The **Gel**.sub.C247 at 77 mg/mL was diluted with in 10 mM Na Phosphate, 0.1 M NaCl to a concentration of 5 mg/mL,. . . 0.2 M NaCl,

pH 7.5. Free thiol content was determined to be 1.48 moles of free SH per mole of **Gel**.sub.C247 using the DTNB assay. The **Gel** .sub.C247 was concentrated to 10 mg/mL prior to conjugation with activated hel Fab'-M2IT-TNB.

DETD For the reaction between the free thiol on **Gel**.sub.C247 and the derivitized hel Fab'-M2IT-TNB, conditions were as follows. A 5.7-fold molar excess of gelonin was added to activated hel. . . with

1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction solution was loaded onto a **gel** filtration column (AcA54) equilibrated with 10 mM Tris, 250 mM NaCl, pH 7.5. The first peak off this column was. . .

DETD TABLE 2

Conjugate	IC.sub.50 (pM T) HSB2 Cells	PBMCs
H65-RTA	143	459
H65-(M2IT)-S-S-(M2IT)-Gelonin	1770	81
H65-(M2IT)-S-S-(M2IT)-rGelonin	276	75
H65-(M2IT)-S-S-**Gel**.sub.C10	140	28
H65-(M2IT)-S-S-**Gel**.sub.C44	99	51
H65-(M2IT)-S-S-**Gel**.sub.C239	2328	180
H65-(M2IT)-S-S-**Gel**.sub.C244	>5000	>2700
H65-(M2IT)-S-S-**Gel**.sub.C247	41	35
H65-(M2IT)-S-S-**Gel**.sub.C248	440	203
cH65-RTA.sub.30	60	400
cH65-(M2IT)-S-S-(M2IT)-Gelonin	1770	140
cH65-(M2IT)-S-S-(M2IT)-rGelonin	153	120
cH65-(M2IT)-S-S-**Gel**.sub.C239	>7000	290
cH65-(M2IT)-S-S-**Gel**.sub.C247	34	60
cH65-(M2IT)-S-S-**Gel**.sub.C248	238	860

DETD . . . analog conjugates were at least as active as native and recombinant gelonin. Importantly, however, some of the conjugates (such as **Gel**.sub.C10 **Gel**.sub.C10, and **Gel** .sub.C247) exhibited an enhanced potency against PBMCs, and also exhibited an enhanced level of cell kill (data not shown).

DETD TABLE 3

Conjugate	IC.sub.50 (pM T) HSB2 Cells

		PBMCs
cFab'-RTA 30	530	1800
cFab'-rGelonin	135	160
cFab'-**Gel**.sub.C247	48	64
cF(ab').sub.2 -RTA 30	33	57
cF(ab').sub.2 -rGelonin	55	34
cF(ab').sub.2 -**Gel**.sub.C247	23	20
cF(ab').sub.2 -**Gel**.sub.C248	181	95

DETD TABLE 4

Conjugate	IC.sub.50 (pM T) HSB2 Cells	Extent of Kill
he1 Fab'-**Gel**.sub.C247	57.7	93%
he1 Fab-**Gel**.sub.247	180	94%
cFab'-**Gel**.sub.C247	47.5	93%
cF(ab').sub.2 -rGelonin	45.4	85%
mF(ab').sub.2 -**Gel**.sub.C247	77.5	83%
cF(ab').sub.2 -**Gel**.sub.C247	23.2	85%

DETD The cFab'-**Gel**.sub.247 immunoconjugate is clearly more cytotoxic than cFab' conjugates with recombinant gelonin or RTA 30.

DETD TABLE 5

Conjugate	RC.sub.50 (mM)
H65-RTA 30	3.2
H65-(M2IT)-S-S-(M2IT)-gelonin	11.1
H65-(M2IT)-S-S-(M2IT)-rGelonin	3.0
H65-(M2IT)-S-S-**Gel**.sub.C.sub.10	2.5
H65-(M2IT)-S-S-**Gel**.sub.C44	0.6
H65-(M2IT)-S-S-**Gel**.sub.C239	774.0
H65-(M2IT)-S-S-**Gel**.sub.C244	1.2
H65-(M2IT)-S-S-**Gel**.sub.C247	0.1
H65-(M2IT)-S-S-**Gel**.sub.C248	0.4
cH65-RTA 30	2.50
cH65-(M2IT)-S-S-(M2IT)-rGelonin	2.39

cH65-(M2IT)-S-S-**Gel**.sub.C247
0.11
cH65-(M2IT)-S-S-**Gel**.sub.C248
0.32

DETD . . . that the stability of the bonds between the different gelonin proteins and H65 antibody varied greatly. With the exception of **Gel**.sub.C10 and **Gel**.sub.C239, most of the gelonin analogs resulted in conjugates with linkages that were somewhat less stable in this in vitro assay than the dual-linker chemical conjugate. The stability of the **Gel**.sub.C239 analog, however, was particularly enhanced.

DETD TABLE 6

Conjugate	RC.sub.50 (mM)
hel Fab'-**Gel**.sub.C247	0.07
cFab'-Gelonin	1.27
cFab'-**Gel**.sub.C247	0.08
cF(ab').sub.2 -RTA 30	3.69
cF(ab').sub.2 -rGelonin	2.30
cF(ab').sub.2 -**Gel**.sub.C247	0.09
cF(ab').sub.2 -**Gel**.sub.C248	0.32

DETD . . . cut with BamHI and the 760 bp fragment corresponding to amino acids 1-256 of BRIP was purified from an agarose **gel**. Concurrently, a unique XhoI site was introduced downstream of the 3'-end of the BRIP gene in pBS1 by PCR amplification. . . and XhoI, and an 87 bp fragment containing the 3'-end of the BRIP gene was purified on a 5% acrylamide **gel**. The 760 and 87 bp purified BRIP fragments were ligated in the vector pING1500 adjacent to the PelB leader sequence.. . .

DETD . . . was cut with PstI and XhoI, and the BRIP gene linked to the pelB leader was purified from an agarose **gel**. The expression vector pING3217, containing the araB promoter, was cut with PstI and XhoI and ligated to the BRIP gene.. . .

DETD . . . of BRIP with the altered amino acid was excised from pMB2X and the fragment was purified on a 5% acrylamide **gel**. This fragment along with an EcoRI to BamHI fragment containing the pelB leader sequence and sequences encoding the first 256. . .

DETD . . . BRIP analog, was treated with T4 polymerase, cut with XhoI, and the resulting fragment was purified on a 5% acrylamide **gel**. Concurrently, plasmid pING3322 was cut with BamHI, treated with T4 polymerase, cut with EcoRI, and the fragment containing the pelB. . .

DETD . . . and the 51 bp fragment, which encodes the carboxyl terminal portion of the analog, was purified on a 5% acrylamide **gel**. The fragment (corresponding to amino acids 268-276 of BRIP.sub.C270) was cloned in a three piece ligation along with the internal. . .

DETD . . . BRIP-(M2IT)-S-S-TNB was first reduced to BRIP-(M2IT)-SH by treatment with 0.5 mM DTT for 1 hour at 25.degree. C., desalted by **gel** filtration of Sephadex.RTM. GF-05LS to remove the reducing

agent, and then mixed with antibody-(M2IT)-S-S-TNB.

DETD . . . 25.degree. C. to quenched any unreacted m2IT linkers on the antibody. The quenched reaction solution was promptly loaded onto a **gel** filtration column (AcA44) to remove unconjugated ribosome-inactivating protein. Purification was completed using soft **gel** affinity chromatography on Blue Toyopearl.RTM. resin using a method similar to Knowles et al., Analyt. Biochem., 160, 440 (1987). Samples. . .

DETD . . . set out below using IUPAC nucleotide symbols. ##STR16## The resulting 81 bp PCR product was purified on a 5% acrylamide **gel** and cloned into the SmaI site of pUC18. Three candidate clones were sequenced, and one clone, pMO110, was identified which. . .

DETD . . . with momo-9 and momo-10, and the product was treated with T4 polymerase, cut with XhoI, and purified on an agarose **gel**. This gene fragment was ligated along with the 131 bp pelB leader fragment from pIC100 which has been generated by. . .

L9 ANSWER 54 OF 68 USPATFULL

AB The invention provides in vivo methods and compositions for using IL-10 to treat inflammatory bowel disease in a mammal. The method comprises administering to the mammal an effective amount of IL-10, preferably intravascularly, alone or in combination with other therapeutic reagents.

AN 94:104325 USPATFULL

TI Use of IL-10 to treat inflammatory bowel disease

IN Rennick, Donna, Los Altos, CA, United States

PA Schering Corporation, Kenilworth, NJ, United States (U.S. corporation)

PI US 5368854 19941129 <--

AI US 1992-932900 19920820 (7)

DT Utility

FS Granted

EXNAM Primary Examiner: Wityshyn, Michael G.; Assistant Examiner: Sayala, C.

LREP Ching, Edwin P., Dow, Karen B., O'Neal, Lauren C.

CLMN Number of Claims: 25

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 995

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5368854 19941129 <--

SUMM Treatment is similar for both diseases and includes steroids, sulphasalazine and its derivatives, and immunosuppressive drugs such as cyclosporin A, **mercaptopurine** and **azathioprine**.

SUMM . . . one additional therapeutic agent. Examples of such agents include corticosteroids, sulphasalazine, derivatives of sulphasalazine, immunosuppresive drugs such as cyclosporin A, **mercaptopurine**, and **azathioprine**, and another cytokine. The co-administration can be sequential or simultaneous. Co-administration generally means that the multiple (two or more) therapeutics. . .

SUMM . . . tests such as an ultrasonic study or a radiographic test. Some examples of signs of IBD include abdominal mass, glossitis, **aphthous** ulcer, anal fissure, perianal fistula, anemia, malabsorption, and iron deficiency. Occasionally, signs and symptoms overlap. For example, the patient complains. . .

DETD . . . to standard procedures well known in the art. For example, purification steps could include ammonium sulfate precipitation, ion exchange chromatography, **gel** filtration, electrophoresis, affinity chromatography, and the like. See Jakoby (ed.), "Enzyme Purification and Related Techniques," Methods in Enzymology 22:233-577 (1977);. . .

DETD . . . which are isolated by lysing the E. coli cell and centrifuging

the resultant supernatant at about 13,000 g. The resultant **pellet** is collected and washed by homogenizing in an appropriate buffer to remove contaminant proteins.

DETD The synthetic peptides are usually purified by a method such as **gel** filtration chromatography or high performance liquid chromatography. See, for example, Stewart & Young, Solid Phase Peptide Synthesis, Pierce Chemical Company,. . .

DETD . . . therapeutic agents. Examples of such agents include corticosteroids, sulphasalazine, derivatives of sulphasalazine, and selected cytotoxic drugs such as cyclosporin A, **mercaptopurine** , and **azathioprine**. Typically, the multiple medications are separately infused or injected sequentially. In appropriate circumstances, multiple medications are mixed and infused or. . .

DETD . . . which may include diarrhea, abdominal pain, fever, melena, hematochezia, and weight loss and signs which may include abdominal mass, glossitis, **aphthous** ulcer, anal fissure, perianal fistula, anemia, malabsorption, and iron deficiency. The patient is initially treated with five .mu.g of IL-10. . .

CLM What is claimed is:

. . . method of claim 10 wherein the additional therapeutic agent is selected from a group consisting of corticosteroids, sulphasalazine, cyclosporin A, **mercaptopurine**, and **azathioprine**.

25. The composition of claim 18 wherein the sign is selected from a group consisting of abdominal mass, glossitis, **aphthous** ulcer, anal fissure, perianal fistula, anemia, malabsorption, and iron deficiency.

L9 ANSWER 55 OF 68 USPATFULL

AB Microbial transformation of a macrolide immunosuppressant by the microorganism Streptomyces sp., (Merck Culture Collection MA 6960) ATCC No. 55387 yields a compound of the structural formula (I): ##STR1## This compound is an immunosuppressant useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases, the prevention of rejection of foreign organ transplants and/or related afflictions, diseases and illnesses.

AN 94:86509 USPATFULL

TI Microbial transformation product having immunosuppressive activity

IN Shafiee, Ali, Westfield, NJ, United States
Arison, Byron H., Watchung, NJ, United States
Chen, Shieh-Shung T., Morganville, NJ, United States
Miller, Randall R., Piscataway, NJ, United States
Stearns, Ralph A., Park Ridge, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5352783 19941004 <--

AI US 1993-74258 19930609 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Bond, Robert T.

LREP Thies, J. Eric, Rose, David L., DiPrima, Joseph F.

CLMN Number of Claims: 1

ECL Exemplary Claim: 1

DRWN 1 Drawing Figure(s); 1 Drawing Page(s)

LN.CNT 906

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5352783 19941004 <--

SUMM . . . rejection of foreign organ transplants (e.g. bone marrow and

heart transplants and xeno transplants) and is also useful in the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as: psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, Alopecia areata), male pattern alopecia, alopecia senilis, reversible obstructive airways. . .

SUMM . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyperproliferative skin diseases and of cutaneous manifestations of immunologically-mediated illness. A Fisons WIPO patent application (PCT.

. .

DETD . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .

DETD . . . the suppression of in vitro immune systems (J. Antibiotics 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).

DETD . . . anion or cation exchange resin, non-ionic adsorption resin, etc.), treatment with a conventional adsorbent (e.g. activated charcoal, silicic acid, silica **gel**, cellulose, alumina, etc.), crystallization, recrystallization, and the like. A preferred recovery method is solvent extraction, particularly using methanol. A preferred purification method involves the use of chromatography, especially HPLC, using a silica **gel** count and an eluant mixture composed of water and an organic solvent such as methanol, acetonitrile and the like. A. . .

DETD . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne, Alopecia areata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compound of. . .

DETD . . . or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .

DETD . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of: **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester, cyclosporin. and rapamycin.

DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2

minutes at 4.degree. C. Cold medium was added and cells were again.

L9 ANSWER 56 OF 68 USPATFULL
AB O-Heteroaryl, O-alkylheteroaryl, O-alkenylheteroaryl and O-alkynylheteroaryl-macrolides of the general structural Formula I: ##STR1## have been prepared from suitable precursors by alkylation and/or arylation at C-3" and/or C-4" of the cyclohexyl ring. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases, the prevention of rejection of foreign organ transplants and/or related afflictions, diseases and illnesses.
AN 94:82355 USPATFULL
TI O-heteroaryl, O-alkylheteroaryl, O-alkenylheteroaryl and O-alkynylheteroarylmacrolides having immunosuppressive activity
IN Sinclair, Peter J., Highland Park, NJ, United States
Goulet, Joung, Westfield, NJ, United States
Wong, Frederick, Glen Ridge, NJ, United States
Goulet, Mark, Westfield, NJ, United States
Parsons, William H., Rahway, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5349061 19940920 <--
AI US 1993-135200 19931012 (8)
RLI Continuation-in-part of Ser. No. US 1992-921851, filed on 5 Aug 1992, now patented, Pat. No. US 5252732, issued on 12 Oct 1993 which is a continuation-in-part of Ser. No. US 1991-756946, filed on 9 Sep 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Thies, J. Eric, Rose, David L., DiPrima, Joseph F.
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 5454
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5349061 19940920. <--
PARN . . . infectious diseases, the prevention of rejection of foreign organ transplants (e.g. bone marrow and heart transplants and xeno transplants), the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as: psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, Alopecia areata), male pattern alopecia, alopecia senilis, reversible obstructive airways. . .
PARN . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyperproliferative skin diseases and of cutaneous manifestations of immunologically-mediated illness. A Fisons WIPO patent application (PCT.
. .
PARN . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**,

sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .

PARN . . . the supression of in vitro immune systems (J. Antibiotics 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).

PARN . . . 3,382,247, 3,644,364 and 4,098,791. Upjohn United States Patents (U.S. Pat. Nos. 4,139,619and 4,596,812) discloses the use of minoxidil in the **topical** treatment of human baldness. Similarly, an Upjohn United States Patent (U.S. Pat. No. 5,026,691) discloses the use of minoxidil and an antiinflammatory agent for the treatment of patterned male and female alopecia. Japanese patent Kokai 61-260010 states that **topical** minoxidil formulations containing other specified agents may be prepared. An Upjohn WIPO patent application (PCT Publication No. WO 92/09259) discloses. . . University of Miami WIPO patent application (PCT Publication No. WO 92/12703) discloser a method of stimulating hair growth comprising the **topical** application of a phospholipid.

PARN . . . chloroform, benzene, toluene and the like. The triheteroarylbismuth(V) reagent can be used without purification or can be purified by silica **gel** chromatography. Triheteroarylbismuthines may be prepared by the reaction of an appropriate heteroaryl Grignard reagent or lithiated heteroaryl species with bismuth. . .

PARN . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne Alopecia areata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .

PARN . . . or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .

PARN . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of **azathioprine** (AZA), brequinar sodium, deoxyspergualin (DSG), mizaribine, mycophenolic acid morpholino ester (RS-61443), cyclosporin and rapamycin.

PARN . . . with water and saturated sodium chloride solution, dried with anhydrous magnesium sulfate and concentrated. The residue was chromatographed on silica **gel** (66% ethyl acetate: 33% hexane: 1% methanol) to give 350 mg of product. This material was dissolved in 10 ml. . .

PARN . . . under a nitrogen atmosphere. The solvent was removed under reduced pressure and the dark residue was purified by chromatography (silica **gel**, 7% i-propanol/CH.sub.2 Cl.sub.2) to give 17-ethyl-1-hydroxy-12-[2'-(4"-hydroxy-3"-isopropyloxy-cyclohexyl) -1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo -[22.3.1.0.sup.4,9]octacos-14,18-diene-2,3,10,16-tetraone (180 mg) as a white solid. This material was dissolved in. . . introduced via balloon for 30 min. and the mixture was filtered through celite. Removal of solvent followed by chromatography (silica **gel**) gave 172 mg of the title compound. Mass, .sup.1 H and

.sup.13 C NMR data were consistant with the title. . .

PARN . .. . layer was washed (water, sat'd NaHCO.sub.3, sat'd NaCl) and dried (anhydrous MgSO.sub.4). Removal of solvent followed by chromatography on silica **gel** (70% hexane/ethyl acetate) gave 150 mg of product.

PARN . . . sodium bicarbonate and extracted with ethyl acetate three times. Normal work-up and removal of solvent followed by purification on

silica **gel** column (80% ethyl acetate/hexane) gave 560 mg of the product (2a) as a white solid. MASS: (FAB) 954 (M.sup.+ +Li).

PARN . . . quenched with saturated sodium bicarbonate, then extracted with

ethyl acetate. Removal of solvent in vacuo followed by chromatography on

silica **gel** (80% ethyl acetate/hexane) gave 300 mg of product (Mass, .sup.1 H and .sup.13 C NMR data consistent with the title. . .

PARN . . . with brine and the organic phase dried over magnesium sulfate. Removal of solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (235 mg).

PARN . . . acetate, washed with brine and dried over magnesium sulfate. The solution was concentrated and purified by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) to give the title compound (89 mg). (.sup.1 H NMR consistent with the desired structure).

PARN . . . was warmed to room temperature. Extraction from ethyl acetate, drying over magnesium sulfate and purification by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% MeoH) gave the title compound (22 mg). (.sup.1 H NMR consistent with the desired structure).

PARN . . . the organic phase dried by passage through a magnesium sulfate column. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (2:1)+1% methanol) gave the title compound.

PARN . . . the organic phase dried by passage through a magnesium sulfate column. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compound.

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (20 mg). MAS: (FAB) 878 (M+Li). Partial .sup.1 H NMR .delta.:. . .

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (16 mg 4" ether; 13 mg 3" ether).

DETD . . . with Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC 3.times. on silica **gel** (3:1, hexane/acetone) to give 23 mg of 17-ethyl-1,14-dihydroxy- 12-[2'-(4"-(benzothien -2-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.

DETD . . . with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The

product was isolated and purified by preparative TLC 2.times. on silica **gel** (2:1, hexane/acetone) to give 36 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(thien-2-yl)oxy-3"-methoxycyclo -hexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-

dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone.

DETD . . . brine. The organic phase was dried over magnesium sulfate. Removal of the solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate: hexane (1:6)+1% methanol) gave the title compound (2.37 g). .sup.1 H NMR consistent with the desired structure.

DETD . . . with saturated sodium bicarbonate and brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (2.1 g). .sup.1 H NMR consistent with the desired structure.

DETD . . . water and brine. The organic layer was dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:5))+1% methanol) gave the title compound (1.03 g). .sup.1 H NMR consistent with the desired structure.

DETD . . . (2.times.), saturated sodium bicarbonate and brine and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (705 mg). 1 H NMR consistent with the desired structure.

DETD . . . portion was washed with saturated sodium bicarbonate and brine,

dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (1.45 g). .sup.1 H NMR consistent with the desired structure.

DETD . . . combined organic portion was washed with brine. This was dried over magenesium sulfate and purified by flash chromatography on silica **gel** (2% methanol in methylene chloride followed by 2% methanol in methylene chloride+0.5% acetic acid) to give the title compound (255.

. .

DETD . . . sodium bicarbonate and brine, respectively. The organic portion

was dried over magnesium sulfate and purified by flash chromatography on

silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (138 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . water and brine. The organic layer was dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:9))+1% methanol) gave the title compound (434 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . (2.times.), saturated sodium bicarbonate and brine and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (3:1)+1% methanol) to give the title compound (177 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . portion was washed with saturated sodium bicarbonate and brine,

dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:3)+1% methanol) to give the title compound (157 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . organic portion was washed with brine. It was dried over magenesium sulfate and purified by f lash chromatography on silica **gel** (2% methanol in methylene chloride followed by 2% methanol in methylene chloride+0.5% acetic acid) to give the title compound (114.

. .

DETD . . . sodium bicarbonate and brine, respectively. The organic portion

was dried over magnesium sulfate and purified by flash chromatography on

silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (28 mg). MASS (FAB) 1041 (M+Li); Partial .sup.1 H NMR

.delta.:. . .

DETD . . . sodium bicarbonate and brine, respectively. The organic portion

was dried over magnesium sulfate and purified by flash chromatography on

silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (7.5 mg). MASS (FAB) 983 (M+Li); partial .sup.1 H NMR

.delta.:. . .

DETD . . . combined, dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by flash column chromatography on silica **gel** (3:1 hexane/acetone) followed by preparative TLC (3% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 111 mg of the title compound.. . .

DETD . . . dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (2:1 hexane/acetone then 5% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 10.2 mg of the title compound. Partial .sup.1 H. .

.

DETD . . . filtered, and concentrated in vacuo to a brown oil. The product

was isolated and purified by preparative TLC on silica **gel** (first with 2:1 hexane/acetone followed by 3% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 60 mg of the title compound.. . .

DETD . . . with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The

product was isolated and purified by preparative TLC 3.times. on silica **gel** (2:1, hexane/acetone; 3% CH.sub.3 OH in CH.sub.2 Cl.sub.2 ; 2:1, hexane/acetone) to give 70 mg of the title compound. MASS. . .

DETD . . . filtered and concentrated in vacuo. The product is isolated and

purified from the C-3" ether by preparative TLC on silica **gel** to give the title compound.

DETD . . . combined, dried over anhydrous MgSO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica **gel** (4:1 hexanes/acetone) affording 730 mg 1-allyl-5-bromoindole.

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) followed by preparative TLC (3.5% methanol/CH.sub.2 Cl.sub.2) affording 56 mg pure title compound. MASS (FAB) M+Li 953. Partial. . .

DETD . . . over anhydrous NaSO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by flash column chromatography on silica **gel** (3:1 hexanes/acetone) followed by preparative TLC (3.5% methanol/CH.sub.2 Cl.sub.2 affording 163 mg pure 17-ethyl-1,14-dihydroxy-12-[2'-(4"-1'-allylindol-5'yl)oxy-3"-hydroxy cyclohexyl]-1'-methylvinyl]-23,25-dimethoxy-13,19,21, 27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9] octacos-18-ene-2,3,10,16-tetraone. MASS (FAB),. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by flash column

chromatography on silica **gel** (3:1 hexanes/acetone) followed by preparative TLC (3.5% methanol/CH.sub.2 Cl.sub.2) affording 100 mg of the title compound. MASS (FAB) M+Li 977.. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by flash column chromatography on silica **gel** (3:1 hexanes/acetone) followed by preparative TLC (3.5% methanol/CH.sub.2 Cl.sub.2) affording 100 mg of the title compound. MASS (FAB) M+Li 1003.. . .

DETD . . . organic layer was washed with brine then dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:4)+1% methanol) gave the title compound (230 mg; trichloroacatamide present). .sup.1 H NMR consistent with the desired. . .

DETD . . . the organic layer was washed with brine and dried over magnesium sulfate. Purification of the concentrate-by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)) gave the title compound (42 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . with saturated sodium bicarbonate and brine then dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3) +1% methanol) gave the title compound (25 mg). MASS (FAB) 938 (M+Li); Partial .sup.1 H NMR .delta.:. . .

DETD . . . organic layer was washed with brine then dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate : hexane (1:1)+1% methanol) gave the title compound (42 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . with saturated sodium bicarbonate and brine then dried over magnesium sulfate. Purification of the concentrate by preparative TLC on

silica **gel** (ethyl acetate:hexane (1:1+1% methanol) gave the title compound (9 mg). MASS (FAB) 956 (M+Li) Partial .sup.1 H NMR .delta.: 7.19. . .

DETD . . . organic layer was washed with brine then dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:5)+1% methanol) gave the title compound (150 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . organic layer was washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (63 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . with saturated sodium bicarbonate and brine then dried over magnesium sulfate. Purification of the concentrate by preparative TLC on

silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (26 mg). Partial .sup.1 H NMR 6: 7.18 (s, 1H); 7.16 (d,. . .

DETD . . . dried over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo The product was isolated and purified by preparative TLC on silica

gel (3:1, hexane/acetone) to give 144 mg. of the title compound.

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 81 mg of the title compound. Partial .sup.1 H NMR (CDCl.sub.3, 200 MHz) d: 7.22 (d,. . .

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 44.8 mg of the title compound. Partial .sup.1 H NMR (CDCl, 200 MHz) d: 7.24 (d,. . .

DETD . . . dried over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (3:1, hexane/acetone) to give 150 mg. of the title compound.

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 55 mg of the title compound.

DETD . . . dried over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo The product was isolated and purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 76 mg. of the title compound. Partial .sup.1 H NMR (CDCl, 400 MHz) d: 7.44 (d,. . .

DETD . . . the ice bath, and stirred at room temperature for 2h. The crude reaction mixture was loaded directly onto the silica **gel** column and purified (ethyl acetate:hexane (2:3)+1% MeOH) to give the title compound (197 mg). Partial .sup.1 H NMR (CDCl.sub.3)d: 7.29. .

DETD . . . dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) and again (3.5% CH.sub.3 OH/CH.sub.2 Cl.sub.2) giving 78 mg 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(1-methyl-3-phenylindol-5-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy 13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 1003 (M.sup.+ +Li); 996. . .

DETD . . . dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) giving 200 mg. 17 -ethyl-1,14-dihydroxy-12-[2'-(4"-(1-methyl-3-(2-t-butyldimethyl -silyloxyethyl)indol-5-yl)oxy-3"-methoxycyclohexyl)-1 '-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl -11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene -2,3,10,16-tetraone as a brown oil.

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (1:1 hexanes/acetone) and again (7% CH.sub.3 OH/CH.sub.2 Cl.sub.2) giving 75 mg. 17-ethyl-1, 14-dihydroxy -12-[2'-(4.increment.-(1-methyl-3-(2-hydroxyethyl)indol-5-yl)oxy -3"-methoxy-cyclohexyl)-1 '-methylvinyl]- 23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.

DETD . . . The organic extracts were combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. Loaded residue onto a silica **gel** plug in a fritted filter and eluted with 4:1 hexanes/acetone. Collected fractions containing the desired product and concentrated in vacuo. . .

DETD . . . dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) and again (3.5% CH.sub.3 OH/CH.sub.2 Cl.sub.2) and again (2:1 hexanes/acetone) giving 253 mg. 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(1-benzylindol-5-yl)oxy-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.

DETD . . . dried over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on

silica **gel** (3:1,hexane/acetone) to give 318 mg of the title compound.

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1,hexane/acetone) to give 190 mg of the title compound.

DETD . . . ethyl acetate, washed with 1N HCl, saturated NaHCO.sub.3, and brine. The product was purified by flash column chromatography on silica **gel** (5% methanol/CH.sub.2 Cl.sub.2 and then 5% methanol/CH.sub.2 Cl.sub.2 plus 1% NH.sub.4 OH) to give 74 mg. Mass (FAB) 1064 (M.sup.+. . .

DETD . . . ethyl acetate, washed with 1N HCl, saturated NaHCO.sub.3, and brine. The product was purified by flash column chromatography on silica **gel** (45/65 acetone/hexanes) to give 50 mg. 17-Ethyl-1,14dihydroxy-12-[2'-(4"-(1'"-(2""-(2'""-hydroy) -ethylaminocarbonyloxy)ethyl)indol-5'"-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]- 23,25-dimethoxy -13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 1061 (M.sup.+ +Na); 1038 (M.sup.+ +1).

DETD . . . diluted with ethyl acetate, washed with 1N HCl and brine. The product was purified by flash column chromatography on silica **gel** (2:3 acetone/hexanes) to give 50 mg. 17-Ethyl -1,14-dihydroxy-12-[2'-(4"-(1'"-(2""-(isopropyamino - carbonyloxy)ethyl)indol-5'"-yl)oxy-3"-methoxycyclohexyl) -1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 1043 (M.sup.+ +Li).

DETD . . . diluted with ethyl acetate, washed with 1N HCl and brine. The product was purified by flash column chromatography on silica **gel** (4:1 hexanes/ acetone) to give 115 mg. 17-Ethyl-1,14-dihydroxy -12-[2'-(4"-(1"'-(2""-(1""'-piperidinocarbonyl -oxy)ethyl)indol-5"'-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl -11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene -2,3,10,16-tetraone. Mass (FAB) 1062 (M.sup.+).

DETD . . . ethyl acetate, washed with 1N HCl, saturated aqueous NaHCO.sub.3 and brine. The product was purified by preparative TLC on silica **gel** (4% MeOH/CH.sub.2 Cl.sub.2) to give 85 mg. product. The compound was further purified by preparative TLC on silica **gel** (4% MeOH/CH.sub.2 Cl.sub.2) to give 67 mg. 17-Ethyl -1,14-dihydroxy-12-[2'-(4"-(1"'-(2""(1'""-morphilinocarbonyloxy)ethyl)in dol-5"'-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13, 19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 1064 (M.sup.+).

DETD . . . were dried over anhydrous MgSO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) giving 310 mg. 17-Ethyl-1,14-dihydroxy-12-[2'-(4"-1"'-(2""-azidoethyl)indol-5"'-yl)oxy-3"-methoxy -cyclohexyl)-1 '-methylvinyl]-23,25-dimethoxy-13,19,21, 27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 975 (M.sup.+).

DETD . . . temperature for 16 hours The solvent was removed in vacuo. The product was purified by flash column chromatography on silica

gel (10% MeOH/CH.sub.2 Cl.sub.2) giving 227 mg. 17-Ethyl-1,14-dihydroxy-12-[2'-(4"-(1"'-(2""-aminoethyl)indol -5"'-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23, 25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 956(M.sup.+ +Li).

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 51 mg of the title compound. Partial .sup.1 H NMR (CDCl.sub.3, 200 MHz) .delta.:7.19 (d, J=9. . .

DETD . . . which was then dried with Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was purified by column chromatography (silica **gel**, 4:1 hexane/acetone) to give 457 mg. of the title compound. Partial .sup.1 H NMR (CDCl.sub.3, 200 MHz) .delta.:9.77 (s, 1.

. .

DETD a **cream** is prepared from A phase and B phase having the following compositions.

DETD . . . B phase is added to the A phase followed by stirring, and the obtain emulsion is cooled to obtain a **cream**.

L9 ANSWER 57 OF 68 USPATFULL

AB Imidazolidyl macrolides of the general structural Formula I: ##STR1## have been prepared from suitable precursors by alkylation and/or arylation at C-3" and/or C-4" of the cyclohexyl ring. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases the prevention of rejection of foreign organ transplants and/or related afflictions, diseases and illnesses.

AN 94:77811 USPATFULL

TI Imidazolidyl macrolides having immunosuppressive activity

IN Goulet, Mark, Westfield, NJ, United States
Sinclair, Peter J., Highland Park, NJ, United States
Wong, Frederick, Glen Ridge, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5344925 19940906 <--

AI US 1993-124137 19930920 (8)

RLI Continuation-in-part of Ser. No. US 1992-921181, filed on 4 Aug 1992, now patented, Pat. No. US 5247076, issued on 21 Sep 1993 which is a continuation-in-part of Ser. No. US 1991-756633, filed on 9 Sep 1991, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Bond, Robert T.

LREP Thies, J. Eric, Rose, David L., DiPrima, Joseph F.

CLMN Number of Claims: 7

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 3206

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5344925 19940906 <--

SUMM . . . of foreign organ transplants, (e.g. bone marrow, kidney, liver, heart, skin, small-bowel, and pancreatic islet-cell transplants, including xeno transplants), the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as:

psoriasis, atopical dermatitiis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus or Alopecia arcata), male pattern

SUMM . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyperproliferative skin diseases and of cutaneous manifestations of immunologically-mediated illness. A Fisons WIPO patent application (PCT.

. .

SUMM . . . onset diabetes, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Chrons disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .

SUMM . . . the suppression of in vitro immune systems (J. Antibiotics 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).

SUMM . . . . 3,382,247, 3,644,364 and 4,098,791. Upjohn U.S. Pats. (U.S. Pat. Nos. 4,139,619 and 4,596,812) discloses the use of minoxidil in the

topical treatment of human baldness. Similarly, an Upjohn U.S. Pat. (U.S. Pat. No. 5,026,691) discloses the use of minoxidil and an antiinflammatory agent for the treatment of patterned male and female alopecia. Japanese patent Kokai 61-260010 states that **topical** minoxidil formulations containing other specified agents may be prepared. An Upjohn WIPO patent application (PCT Publication No. WO 92/09259) discloses. . . University of Miami WIPO patent application (PCT Publication No. WO 92/12703.) discloser a method of stimulating hair growth comprising the **topical** application of a phospholipid.

DETD . . . chloroform, benzene, toluene and the like. The triarylbismuth(V) reagent can be used without purification or can be purified by silica **gel** chromatography. Triarylbismuthines may be prepared by the reaction of an appropriate aryl Grignard reagent with

bismuth trichloride in an inert. . .

DETD . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne Alopecia arcata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .

DETD . . . parenteral applications. The active ingredient may be compounded, for example, with the usual non- toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carders which can be used are water,. . .

DETD . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of **azathioprine** (AZA), brequinar sodium, deoxyspergualin (DSG), mizaribine, mycophenolic acid morpholino ester CRS-61443), cyclosporin and rapamycin.

DETD . . . room temperature. After 15 hours, the solution was concentrated
in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound
(45 mg).

DETD . . . room temperature. After 5 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (45 mg). (.sup.1 H NMR consistent with the desired structure).

DETD . . . room temperature. After 4 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound
(20 mg).

DETD . . . room temperature. After 5 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (4:1)+1% methanol) to give the title compound (54.7 mg).

DETD . . . room temperature. After 4 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (10 mg).

DETD . . . room temperature. After 5 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2: 1)+1% methanol) to give the title compound (45 mg).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (112 mg)

DETD . . . extracted with half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (86 mg).

DETD . . . room temperature. After 4 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (7 mg).

DETD . . . diluted with 1 ml ethyl acetate and filtered through diatomaceous earth. The concentrate was purified by flash chromatography
on silica **gel** (ethyl acetate:hexane (4:1)+1% methanol) to give the title compound (4.5 mg).

DETD . . . diluted with 1.5 ml ethyl acetate and filtered through diatomaceous earth. The concentrate was purified by flash chromatography
on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then (4:1)+1% methanol) to give the title compound (10 mg)

DETD . . . diluted with 1.5 ml ethyl acetate and filtered through diatomaceous earth. The concentrate was purified by flash chromatography
on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then (4:1)+1% methanol) to give the title compound (9 mg)

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on
silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the

title compound (165 mg)

DETD . . . washed with brine. The combined organics were dried over magnesium sulfate and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) to give the title compound (79 mg)

DETD . . . washed with brine, dried over magnesium sulfate and concentrated in vacuo. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (68.4 mg).

DETD . . . room temperature. After 20 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound (20 mg)

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (156 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (21 mg 4"-ether; 17 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (15 mg 4"-ether; 16 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compound (12 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compounds (11 mg 4"-ether; 13 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (6.8 mg).

DETD . . . brine and the organic phase dried over magnesium sulfate. Removal of the solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate: hexane (1:3)+1% methanol) gave the title compound (2.91 g).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:1)+1% methanol) gave the title compound (1.51 g).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ether:hexane (2:3)) gave the title compound (800 mg).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride:hexane:methanol (10:2:1)) to give the title compound (300 mg).

DETD . . . from half-saturated sodium bicarbonate. The organic portion was dried over magnesium surf ate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (151 mg).

DETD . . . bicarbonate solution and the organic phase is dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** gives the title compound.

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (320 mg).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride: hexane:methanol (10:2:1)) to give the title compound (232 mg).

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (112 mg).

DETD . . . was extracted with ethyl acetate (3.times.15ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1+1% methanol) to give the title compound (80.2 mg).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then (4:1)+1% methanol) to give the title compound (680 mg).

DETD . . . over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The product was isolated and purified by preparative tlc on silica **gel** (3:1, hexane/acetone) to give the desired product (105mg).

DETD . . . washed with brine. The combined organics were dried over magnesium sulfate and the concentrate purified by preparative tlc on silica **gel** (2: 1, hexane/acetone) to give the title compound (6 mg).

DETD . . . room temperature. After 1.5 hours, the solution was concentrated in vacuo and the mixture purified by preparative tlc on silica **gel** (2:1 hexane/acetone) to give the title compound (20 mg).

DETD . . . room temperature. After 18 hours, the solvent was removed in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (62 mg).

DETD . . . acetonitrile:hexane (3:1). The acetonitrile layer was dried over magnesium surf ate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (58.8 mg).

DETD . . . extracted with chloroform (3.times.60 mL). The combined organics were dried over magnesium sulfate and purified by flash chromatography on silica **gel** (chloroform:methanol:water 40:10:1) to give the title compound (62.5 mg).

DETD . . . N,N-dimethyl-aminopyridine (6.9 mg) and the mixture stirred at room temperature. After 6 hours, the reaction was applied to a silica **gel** column and purified by flash chromatography (5% methanol in methylene chloride) to give 17-ethyl-1-hydroxy-14-(N,N-dimethylaminoacetoxy)-12-[2'-(4"-(4"'-(3"",5""-dimethoxyphenyl)-2"'-imidazolylmethyloxy)-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-

azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone (7 mg). This material was. . .

DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2 minutes at 4.degree. C. Cold medium was added and cells were again. .

L9 ANSWER 58 OF 68 USPATFULL

AB Pharmaceutical compositions comprising antibodies to intercellular adhesion molecule-1 (ICAM-1 or CD54) are useful in methods of decreasing the severity of inflammation associated with the adhesion of leukocytes to cells bearing ICAM-1. Treatment with anti-ICAM-1 antibodies reduced the severity of inflammation associated with acute organ or tissue rejection and prolonged allograft survival time. Such compositions may optionally contain other immunsuppressive agents.

AN 94:11498 USPATFULL

TI Intercellular adhesion molecules, and their binding ligands

IN Springer, Timothy A., Newton, MA, United States
Rothlein, Robert, Danbury, CT, United States
Marlin, Steven D., Danbury, CT, United States
Dustin, Michael L., University City, MO, United States

PA Dana Farber Cancer Institute, Boston, MA, United States (U.S. corporation)

PI US 5284931 19940208 <--

AI US 1990-515478 19900427 (7)

RLI Continuation-in-part of Ser. No. US 1989-456647, filed on 22 Dec 1989 which is a continuation-in-part of Ser. No. US 1987-45963, filed on 4 May 1987 which is a continuation-in-part of Ser. No. US 1987-115798, filed on 2 Nov 1987 which is a continuation-in-part of Ser. No. US 1988-155943, filed on 16 Feb 1988 which is a continuation-in-part of Ser. No. US 1988-189815, filed on 3 May 1988 which is a continuation-in-part of Ser. No. US 1988-250446, filed on 28 Sep 1988 which is a continuation-in-part of Ser. No. US 1989-324481, filed on 16 Mar 1989 which is a continuation-in-part of Ser. No. US 1989-373882, filed on 30 Jun 1989 which is a continuation-in-part of Ser. No. US 1989-456647, filed on 22 Dec 1989

DT Utility

FS Granted

EXNAM Primary Examiner: Nucker, Christine M.; Assistant Examiner: Cunningham, Thomas

LREP Sterne, Kessler, Goldstein & Fox

CLMN Number of Claims: 11

ECL Exemplary Claim: 1

DRWN 26 Drawing Figure(s); 25 Drawing Page(s)

LN.CNT 4753

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5284931 19940208 <--

SUMM (b) at least one immunosuppressive agent selected from the group consisting of: dexamethesone, **azathioprine** and cyclosporin A.

DETD . . . such a screen. Thus, for example, the antigen bound by the antibody may be analyzed as by immunoprecipitation and polyacrylamide **gel** electrophoresis. If the bound antigen is a member of the LFA-1 family of molecules then the immunoprecipitated antigen will be. . .

DETD . . . a Teflon Potter Elvejhem homogenizer, and then centrifuged at 1000 .times.g for 15 minutes. The supernatant was retained and the **pellet** was re-extracted with 200 ml of 2.5% Tween 40 in Tris-saline. After centrifugation at 1000 .times.g for 15 minutes, the

supernatants from both extractions were combined and centrifuged at 150,000 .times.g for 1 hour to **pellet** the membranes. The membranes were washed by resuspending in 200 ml Tris-saline, centrifuged at 150,000 .times.g for 1 hour. The membrane **pellet** was resuspended in 200 ml Tris-saline and was homogenized with a motorized homogenizer and Teflon pestle until the suspension was. . .

DETD . . . be used in structural studies, a column of 10 ml of RR1/1-Sepharose CL-4B (coupled at 2.5 mg of antibody/ml of **gel**), and two 10 ml pre-columns of CNBr-activated, glycine-quenched Sepharose CL-4B, and rat-IgG coupled to Sepharose CL-4B (2mg/ml) were used. The. . .

DETD Approximately 200 .mu.g of purified ICAM-1 was subjected to a second stage purification by preparative SDS-polyacrylamide **gel** electrophoresis. The band representing ICAM-1 was visualized by soaking the **gel** in 1 M KCl. The **gel** region which contained ICAM-1 was then excised and electroeluted according to the method of Hunkapiller et al., Meth. Enzymol. 91:227-236. . .

DETD ICAM-1 purified from human spleen migrates in SDS-polyacrylamide **gels** as a broad band of M.sub.r of 72,000 to 91,000. ICAM-1 purified from JY cells also migrates as a broad. . .

DETD . . . to Eco R1 linkers (New England Biolabs), digested with Eco R1 and size selected on a low melting point agarose **gel**. cDNA greater than 500bp were ligated to .lambda.gt10 which had previously been Eco R1 digested and dephosphorylated (Stratagene) The product. .

DETD . . . the manufacturers recommended quantity of Bam H1 and Eco R1 endonucleases (New England Biolabs). Following electrophoresis through a 0.8% agarose **gel**, the DNAs were transferred to a nylon membrane (Zeta Probe, BioRad). The filter was prehybridized and hybridized following standard procedures. . . 20 .mu.g of total RNA or 6 .mu.g of poly(A).sup.+ RNA. RNA was denatured and electrophoresed through a 1% agarose-formaldehyde **gel** and electrotransferred to Zeta Probe. Filters were prehybridized and hybridized as described previously (Staunton, D. E., et al. Embo J.. . .

DETD . . . diseases were studied for their expression of ICAM-1 and HLA-DR. A proportion of keratinocytes in biopsies of allergic contact eczema, **pemphigoid**/pemphigus and lichen planus expressed ICAM-1. Lichen planus biopsies showed the most intense staining with a pattern similar to or even. . .

DETD . . . No. of ICAM-1 HLA-DR ICAM-1 &

Diagnosis	Cases	Only	Only	HLA-DR
Allergic Contact	5	.sup. 3.sup.a	0	2
Eczema				
Lichen Planus	11	3	0	8
Pemphigoid/ Pemphigus	2	2	0	0
Exanthema	3	2	0	0
Urticaria	4	1	0	1

.sup.a Samples were considered as positive if at. . .

DETD . . . and anti-LFA-1 antibodies. In order to determine whether the combined administration of anti-ICAM-1 and other immunosuppressive agents (such as dexamethasone, **azathioprine**, cyclosporin A or

steroids (such as, for example, preunisone, etc.) would also have enhanced effects, MLR assays were performed using. . .

DETD . . . the inhibitory effects of R6-5-D6 are at least additive with the inhibitory effects of suboptimal doses of dexamethasone (Table 19), **Azathioprine** (Table 20) and cyclosporin A (Table 21). This implies that anti-ICAM-1 antibodies can be effective in lowering the necessary doses. . .

DETD TABLE 20

Effect of Anti-ICAM-1 and **Azathioprine** on the Human MLR

Group	Inhibitor (ng/ml)	.sup.3 HT Incorporation (CPM)	% Inhibition
Media	--	78	--
Stimulators (S)	--	174	--
Responders (R)	--	3,419	--
R .times. S	--	49,570	--
R .times. S	R6-5-D6 (8)	44,374	11
R .times. S	**Azathioprine** (1)	42,710	14
R .times. S	R6-5-D6 (8) + Azathioprine (1)	34,246	31

CLM What is claimed is:

2. The pharmaceutical composition of claim 1 wherein said immunosuppressive agent is selected form the group consisting of dexamethasone, **azathioprine** and cyclosporin A.

L9 ANSWER 59 OF 68 USPATFULL

AB O-Heteroaryl, O-alkylheteroaryl, O-alkenylheteroaryl and O-alkynylheteroarylmacrolides of the general structural Formula I: ##STR1## have been prepared from suitable precursors by alkylation and/or arylation at C-3" and/or C-4" of the cyclohexyl ring. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases, the prevention of rejection of foreign organ transplants and/or related afflictions, diseases and illnesses.

AN 93:85282 USPATFULL

TI D-heteroaryl, O-alkylheteroaryl, O-alkenylheteroaryl and O-alkynylheteroarylmacrolides having immunosuppressive activity

IN Sinclair, Peter J., Highland Park, NJ, United States
Goulet, Joung, Westfield, NJ, United States
Wong, Frederick, Glen Ridge, NJ, United States
Goulet, Mark, Westfield, NJ, United States
Parsons, William H., Rahway, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States

PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

PI US 5252732 19931012 <--

AI US 1992-921851 19920805 (7)

RLI Continuation-in-part of Ser. No. US 1991-756946, filed on 9 Sep 1991,

now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Caruso, Charles M., Thies, J. Eric
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 6683
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5252732 19931012 <--
SUMM . . . rejection of foreign organ transplants (e.g. bone marrow and heart transplants and xeno transplants) and are also useful in the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as: psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, Alopecia areata), male pattern alopecia, alopecia senilis, reversible obstructive airways. . .
SUMM . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyperproliferative skin diseases and of cutaneous manifestations of immunologically-mediated illness. A Fisons WIPO patent application (PCT.
. .
SUMM . . . diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .
SUMM . . . the suppression of in vitro immune systems (J. Antibiotics 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).
DETD . . . chloroform, benzene, toluene and the like. The triheteroarylbismuth(V) reagent can be used without purification or can be purified by silica **gel** chromatography.
Triheteroarylbismuthines may be prepared by the reaction of an appropriate heteroaryl Grignard reagent or lithiated heteroaryl species with bismuth. . .
DETD . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne, Alopecia areata,
eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .
DETD . . . or parenteral applications. The active ingredient may be compounded, for example, with the usual nontoxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water, . . .
DETD . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent

selected from the group consisting of: **azathioprine**, brequinar sodium, deoxyspergualin, mizaribine, mycophenolic acid morpholino ester, cyclosporin, and rapamycin.

DETD . . . with water and saturated sodium chloride solution, dried with anhydrous magnesium sulfate and concentrated. The residue was chromatographed on silica **gel** (66% ethyl acetate: 33% hexane: 1% methanol) to give 350 mg of product. This material was dissolved in 10 ml. . .

DETD . . . under a nitrogen atmosphere. The solvent was removed under reduced pressure and the dark residue was purified by chromatography (silica **gel**, 7% i-propanol/CH.sub.2 Cl.sub.2) to give 17-ethyl-1-hydroxy-12-[2'-(4"-hydroxy-3"-isopropyloxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-14,18-diene-2,3,10,16-tetraone (180 mg) as a white solid. This material was dissolved in ethanol (20. . . introduced via balloon for 30 min. and the mixture was filtered through celite. Removal of solvent followed by chromatography (silica **gel**) gave 172 mg of the title compound. Mass, .sup.1 H and .sup.13 C NMR data were consistent with the title. . .

DETD . . . layer was washed (water, sat'd NaHCO.sub.3, sat'd NaCl) and dried (anhydrous MgSO.sub.4). Removal of solvent followed by chromatography on silica **gel** (70% hexane/ethyl acetate) gave 150 mg of product. MASS: (FAB) 1110 (M.sup.+ +Li).

DETD . . . sodium bicarbonate and extracted with ethyl acetate three times. Normal work-up and removal of solvent followed by purification on silica **gel** column (80% ethyl acetate/hexane) gave 560 mg of the product (2a) as a white solid. MASS: (FAB) 954 (M.sup.+ +Li).

DETD . . . quenched with saturated sodium bicarbonate, then extracted with ethyl acetate. Removal of solvent in vacuo followed by chromatography on silica **gel** (80% ethyl acetate/hexane) gave 300 mg of product (Mass, .sup.1 H and .sup.13 C NMR data consistent with the title. . .

DETD . . . with brine and the organic phase dried over magnesium sulfate. Removal of solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (235 mg).

DETD . . . acetate, washed with brine and dried over magnesium sulfate. The solution was concentrated and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (89 mg).

DETD . . . was warmed to room temperature. Extraction from ethyl acetate, drying over magnesium sulfate and purification by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% MeoH) gave the title compound (22 mg).

DETD . . . the organic phase dried by passage through a magnesium sulfate column. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) gave the title compound.

DETD . . . the organic phase dried by passage through a magnesium sulfate column. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound. MASS: (FAB) 816 (M+Na).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:2)+1% methanol) gave the title compound (20 mg). MAS: (FAB) 878 (M+Li). Partial .sup.1 H NMR .delta.:. . .

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:.hexane (1:1)+1% methanol) gave the title compounds (16 mg 4" ether; 13 mg 3" ether).

DETD . . . with Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC 3.times. on silica **gel** (3:1, hexane/acetone) to give 23 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(benzothien-2-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.

DETD . . . with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by preparative TLC 2.times. on silica **gel** (2:1, hexane/acetone) to give 36 mg of 17-ethyl-1,14-dihydroxy-12-[2'-(4" -(thien-2-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]-octacos-18-ene-2,3,10,16-tetraone.

DETD . . . brine. The organic phase was dried over magnesium sulfate. Removal of the solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate:hexane (1:6)+1% methanol) gave the title compound (2.37 g). .sup.1 H NMR consistent with the desired structure.

DETD . . . with saturated sodium bicarbonate and brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (2.1 g). .sup.1 H NMR consistent with the desired structure.

DETD . . . water and brine. The organic layer was dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:5))+1% methanol) gave the title compound (1.03 g). .sup.1 H NMR consistent with the desired structure.

DETD . . . (2.times.), saturated sodium bicarbonate and brine and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (705 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . portion was washed with saturated sodium bicarbonate and brine, dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (1.45 g). .sup.1 H NMR consistent with the desired structure.

DETD . . . combined organic portion was washed with brine. This was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (2% methanol in methylene chloride followed by 2% methanol in methylene chloride+0.5% acetic acid) to give the title compound (255. . .

DETD . . . sodium bicarbonate and brine, respectively. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (138 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . water and brine. The organic layer was dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate: hexane (1:9))+1% methanol) gave the title compound (434 mg). .sup.1 H NMR consistent with the desired

structure.

DETD . . . (2.times.), saturated sodium bicarbonate and brine and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (3:1)+1% methanol) to give the title compound (177 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . portion was washed with saturated sodium bicarbonate and brine,

dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:3)+1% methanol) to give the title compound (157 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . combined organic portion was washed with brine. It was dried over magenesium sulfate and purified by flash chromatography on silica **gel** (2% methanol in methylene chloride followed by 2% methanol in methylene chloride+0.5% acetic acid) to give the title compound (114.

. . .

DETD . . . sodium bicarbonate and brine, respectively. The organic portion

was dried over magnesium sulfate and purified by flash chromatography on

silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (28 mg). MASS (FAB) 1041 (M+Li); Partial .sup.1 H NMR

.delta.:. . .

DETD . . . sodium bicarbonate and brine, respectively. The organic portion

was dried over magnesium sulfate and purified by flash chromatography on

silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (7.5 mg). MASS (FAB) 983 (M+Li); partial .sup.1 H NMR

.delta.:. . .

DETD . . . combined, dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated by flash column chromatography on silica **gel** (3:1 hexane/acetone) followed by preparative TLC (3% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 111 mg of the title compound.. . .

DETD . . . dried with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by preparative TLC. on silica **gel** (2:1 hexane/acetone then 5% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 10.2 mg of the title compound. Partial .sup.1 H. . .

DETD . . . filtered, and concentrated in vacuo to a brown oil. The product

was isolated and purified by preparative TLC on silica **gel** (first with 2:1 hexane/acetone followed by 3% CH.sub.3 OH in CH.sub.2 Cl.sub.2) to give 60 mg of the title compound.. . .

DETD . . . with Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The

product was isolated and purified by preparative TLC 3.times. on silica **gel** (2:1, hexane/acetone; 3% CH.sub.3 OH in CH.sub.2 Cl.sub.2 ; 2:1, hexane/acetone) to give 70 mg of the title compound. MASS. . .

DETD . . . filtered and concentrated in vacuo. The product is isolated and

purified from the C-3" ether by preparative TLC on silica **gel** to give the title compound.

DETD . . . combined, dried over anhydrous MgSO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column

chromatography on silica **gel** (4:1 hexanes/acetone) affording 730 mg 1-allyl-5-bromoindole.

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) followed by preparative TLC (3.5% methanol/CH.sub.2 Cl.sub.2) affording 56 mg pure title compound. MASS (FAB) M+Li 953. Partial. . .

DETD . . . over anhydrous NaSO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by flash column chromatography on silica **gel** (3:1 hexanes/acetone) followed by preparative TLC (3.5% methanol/CH.sub.2 Cl.sub.2) affording 163 mg pure 17-ethyl-1,14-dihydroxy-12-[2'-(4"-1'-allylindol-5'yl)oxy-3"-hydroxy cyclohexyl]-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetrone.

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by flash column chromatography on silica **gel** (3:1 hexanes/acetone) followed by preparative TLC (3.5% methanol/CH.sub.2 Cl.sub.2) affording 100 mg of the title compound. MASS (FAB) M+Li 977.. . .

DETD . . . anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was isolated and purified by flash column chromatography on silica **gel** (3:1 hexanes/acetone) followed by preparative TLC (3.5% methanol/CH.sub.2 Cl.sub.2) affording 100 mg of the title compound. MASS (FAB) M+Li 1003.. . .

DETD . . . organic layer was washed with brine then dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:4)+1% methanol) gave the title compound (230 mg; trichloroacatamide present). .sup.1 H NMR consistent with the desired structure.

DETD . . . organic layer was washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)) gave the title compound (42 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . with saturated sodium bicarbonate and brine then dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (25 mg). MASS (FAB) 938 (M+Li); Partial .sup.1 H NMR .delta.: 7.19. . .

DETD . . . organic layer was washed with brine then dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (42 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . with saturated sodium bicarbonate and brine then dried over magnesium sulfate. Purification of the concentrate by preparative TLC on

silica **gel** (ethyl acetate:hexane (1:1+1% methanol) gave the title compound (9 mg). MASS (FAB) 956 (M+Li) Partial .sup.1 H NMR .delta.: 7.19. . .

DETD . . . brine. The organic phase was dried over magnesium sulfate. Removal of the solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (293 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . organic layer was washed with brine then dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:5)+1% methanol) gave the title compound (150 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . organic layer was washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the

title compound (63 mg). .sup.1 H NMR consistent with the desired structure.

DETD . . . with saturated sodium bicarbonate and brine then dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (26 mg). partial .sup.1 H NMR .delta.:7.18 (s, 1H); 7.16 (d, J=9. . .

DETD . . . dried over Na.sub.2 SO4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (3:1, hexane/acetone) to give 144 mg. of the title compound.

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 81 mg of the title compound. Partial .sup.1 H NMR (CDCl.sub.3, 200 MHz) d: 7.22 (d,. . .

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 44.8 mg of the title compound. Partial .sup.1 H NMR (CDCl, 200 MHz) d: 7.24 (d,. . .

DETD . . . dried over Na.sub.2 SO4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (3:1, hexane/acetone) to give 150 mg. of the title compound.

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 55 mg of the title compound.

DETD . . . dried over Na.sub.2 SO4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 76 mg. of the title compound. Partial .sup.1 H NMR (CDCl, 400 MHz) d: 7.44 (d,. . .

DETD . . . ice bath, and stirred at room temperature for 2 h. The crude reaction mixture was loaded directly onto the silica **gel** column and purified (ethyl acetate:hexane (2:3)+1% MeOH) to give the title compound (197 mg).

DETD . . . dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) and again (3.5% CH.sub.3 OH/CH.sub.2 Cl.sub.2) giving 78 mg 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(1-methyl-3-phenylindol-5-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.

DETD . . . dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) giving 200 mg. 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(1-methyl-3-(2-t-butyldimethylsilyloxyethyl)indol-5-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup. 4,9]octacos-18-ene-2,3,10,16-tetraone as a brown oil.

DETD . . . combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (1:1 hexanes/acetone) and again (7% CH.sub.3 OH/CH.sub.2 Cl.sub.2) giving -(4"-(1-methyl-3-(2-hydroxyethyl)indol-5-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.

DETD . . . The organic extracts were combined, dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. Loaded residue

onto a silica **gel** plug in a fritted filter and eluted with 4:1 hexanes/acetone. Collected fractions containing the desired product and concentrated in vacuo. . .

DETD . . . dried over anhydrous Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) and again (3.5% CH.sub.3 OH/CH.sub.2 Cl.sub.2) and again (2:1 hexanes/acetone) giving 253 mg. 17-ethyl-1,14-dihydroxy-12-[2'-(4"-(1-benzylindol-5-yl)oxy-3"-hydroxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone.

DETD . . . dried over Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was isolated and purified by preparative TLC on silica **gel** (3:1,hexane/acetone) to give 318 mg of the title compound.

DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1,hexane/acetone) to give 190 mg of the title compound.

DETD . . . ethyl acetate, washed with 1N HCl, saturated NaHCO.sub.3, and brine. The product was purified by flash column chromatography on silica **gel** (5% methanol/CH.sub.2 Cl.sub.2 and then 5% methanol/CH.sub.2 Cl.sub.2 plus 1% NH.sub.4 OH) to give 74 mg. Mass (FAB) 1064 (M.sup.+. . .

DETD . . . ethyl acetate, washed with 1N HCl, saturated NaHCO.sub.3, and brine. The product was purified by flash column chromatography on silica **gel** (45/65 acetone/hexanes) to give 50 mg. 17-Ethyl-1,14-dihydroxy-12-[2'-(4"-(1'"-(2""-(2'""-hydroy)ethylaminocarbonyloxy)ethyl) indol-5'"-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 1061 (M.sup.+ +Na); 1038 (M.sup.+ +1).

DETD . . . diluted with ethyl acetate, washed with 1N HCl and brine. The product was purified by flash column chromatography on silica **gel** (2:3 acetone/hexanes) to give 50 mg. 17-Ethyl-1,14-dihydroxy-12-[2'-(4"-(1'"-(2""-(isopropyaminocarbonyloxy)ethyl)indol-5'"-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 1043 (M.sup.+ +Li).

DETD . . . diluted with ethyl acetate, washed with 1N HCl and brine. The product was purified by flash column chromatography on silica **gel** (4:1 hexanes/acetone) to give 115 mg. 17-Ethyl-1,14-dihydroxy-12-[2'-(4"-(1'"-(2""-(1'""-piperidinocarbonyloxy)ethyl)indol-5'"-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 1062 (M.sup.+).

DETD . . . ethyl acetate, washed with 1N HCl, saturated aqueous NaHCO.sub.3 and brine. The product was purified by preparative TLC on silica **gel** (4% MeOH/CH.sub.2 Cl.sub.2) to give 85 mg. product. The compound was further purified by preparative TLC on silica **gel** (4% MeOH/CH.sub.2 Cl.sub.2) to give 67 mg. 17-Ethyl-1,14-dihydroxy-12-[2'-(4"-(1'"-(2""-(1'""-morphilinocarbonyloxy)ethyl)indol-5'"-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinvyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass

(FAB) 1064 (M.sup.+).
DETD . . . were dried over anhydrous MgSO.sub.4, filtered and concentrated
in vacuo. The product was purified by flash column chromatography on silica **gel** (2:1 hexanes/acetone) giving 310 mg.

17-Ethyl-1,14-dihydroxy-12-[2'-(4"-(1'"-(2""-azidoethyl)indol-5'"-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 975 (M.sup.+).
DETD . . . temperature for 16 hours. The solvent was removed in vacuo. The
product was purified by flash column chromatography on silica **gel** (10% MeOH/CH.sub.2 Cl.sub.2) giving 227 mg.

17-Ethyl-1,14-dihydroxy-12-[2'-(4"-(1'"-(2""-aminoethyl)indol-5'"-yl)oxy-3"-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.0.sup.4,9]octacos-18-ene-2,3,10,16-tetraone. Mass (FAB) 956(M.sup.+ +Li).
DETD . . . were combined, dried over Na.sub.2 SO.sub.4, filtered and concentrated in vacuo. The product was purified by preparative TLC on silica **gel** (2:1, hexane/acetone) to give 51 mg of the title compound. Partial .sup.1 H NMR (CDCl.sub.3, 200 MHz) .delta.:7.19 (d, J=9. . .
DETD . . . which was then dried with Na.sub.2 SO.sub.4, filtered, and concentrated in vacuo. The product was purified by column chromatography
(silica **gel**, 4:1 hexane/acetone) to give 457 mg. of the title compound. Partial .sup.1 H NMR (CDCl.sub.3, 200 MHz) .delta.:9.77 (s, 1H);. . .
DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2 minutes at 4.degree. C. Cold medium was added and cells were again. .

L9 ANSWER 60 OF 68 USPATFULL
AB Imidazolidyl macrolides of the general structural Formula I: ##STR1## have been prepared from suitable precursors by alkylation and/or arylation at C-3" and/or C-4" of the cyclohexyl ring. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases, infectious diseases the prevention of rejection of foreign organ transplants and/or related afflictions, diseases and illnesses.
AN 93:78921 USPATFULL
TI Imidazolidyl macrolides having immunosuppressive activity
IN Goulet, Mark, Westfield, NJ, United States
Sinclair, Peter J., Highland Park, NJ, United States
Wong, Frederick, Glen Ridge, NJ, United States
Wyvratt, Matthew J., Mountainside, NJ, United States
PA Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)
PI US 5247076 19930921 <--
AI US 1992-921181 19920804 (7)
RLI Continuation-in-part of Ser. No. US 1991-756633, filed on 9 Sep 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Bond, Robert T.
LREP Caruso, Charles M., Thies, J. Eric
CLMN Number of Claims: 13

ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 3429
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5247076 19930921 <--
SUMM . . . of foreign organ transplants, (e.g. bone marrow, kidney, liver, heart, skin, small-bowel, and pancreatic islet-cell transplants, including xeno transplants), the **topical** treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (such as: psoriasis, atopical dermatitiis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus or Alopecia areata), male pattern alopecia, alopecia senilis, reversible obstructive. . .
SUMM . . . transplantation. A Sandoz European patent application (EPO Publication No. 0,315,978) discloses the use of FR-900506 and related compounds in the **topical** treatment of inflammatory and hyperproliferative skin diseases and of cutaneous manifestations of immunologically-mediated illness. A Fisons WIPO patent application (PCT. . . . .
SUMM . . . onset diabetes, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Chrons disease, ulcerative colitis, bullous **pemphigoid**, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they. . .
SUMM . . . the supression of in vitro immune systems (J. Antibiotics 1987, 40, 1256). In addition, these compounds are reputed to possess **topical** activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (EPO Pub. No. 0,315,978).
DETD . . . chloroform, benzene, toluene and the like. The triarylbismuth(V) reagent can be used without purification or can be purified by silica **gel** chromatography. Triarylbismuthines may be prepared by the reaction of an appropriate aryl Grignard reagent with bismuth trichloride in an inert. . .
DETD . . . illnesses such as: psoriasis, psoriatic arthritis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous **Pemphigoid**, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, acne Alopecia areata, eosinophilic fasciitis, and atherosclerosis. More particularly, the compounds of. . .
DETD . . . or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, **pellets**, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used are water,. . .
DETD . . . employed in co-therapy with anti-proliferative agents. Particularly preferred is co-therapy with an antiproliferative agent selected from the group consisting of **azathioprine** (AZA), brequinar sodium, deoxyspergualin (DSG), mizaribine, mycophenolic acid morpholino ester (RS-61443), cyclosporin and rapamycin.
DETD . . . room temperature. After 15 hours, the solution was concentrated

in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound (45 mg).

DETD . . . room temperature. After 5 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (45 mg).

DETD . . . room temperature. After 4 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound (20 mg).

DETD . . . room temperature. After 5 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (4:1)+1% methanol) to give the title compound (54.7 mg).

DETD . . . room temperature. After 4 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (10 mg).

DETD . . . room temperature. After 5 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (45 mg).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (112 mg)

DETD . . . extracted with half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (86 mg)

DETD . . . room temperature. After 4 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol) to give the title compound (7 mg).

DETD . . . diluted with 1 ml ethyl acetate and filtered through diatomaceous earth. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (4:1)+1% methanol) to give the title compound (4.5 mg).

DETD . . . diluted with 1.5 ml ethyl acetate and filtered through diatomaceous earth. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then (4:1)+1% methanol) to give the title compound (10 mg)

DETD . . . diluted with 1.5 ml ethyl acetate and filtered through diatomaceous earth. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then (4:1)+1% methanol) to give the title compound (9 mg) (.sup.1 H NMR consistent with the. . .

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (165 mg)

DETD . . . washed with brine. The combined organics were dried over magnesium sulfate and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) to give the title compound (79 mg)

DETD . . . washed with brine, dried over magnesium sulfate and concentrated in vacuo. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (68.4 mg)

DETD . . . room temperature. After 20 hours, the solution was concentrated in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1)+1% methanol, then 2% ammonium hydroxide, 5% methanol in methylene chloride) to give the title compound (20 mg)

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) gave the title compound (156 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (21 mg 4"-ether; 17 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (15 mg 4"-ether; 16 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (12 mg).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by preparative TLC on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compounds (11 mg 4"-ether; 13 mg 3"-ether).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (6.8 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . brine and the organic phase dried over magnesium sulfate. Removal of the solvent in vacuo and flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (2.91 g). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . sodium bicarbonate solution and the organic phase dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) gave the title compound (1.51 g). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ether: hexane (2:3)) gave the title compound (800 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride:hexane:methanol (10:2:1)) to give the title compound (300 mg) (.sup.1 H NMR was consistent. . .

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (151 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . bicarbonate solution and the organic phase is dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** gives the title compound.

DETD . . . combined organics were washed with brine and dried over magnesium sulfate. Purification of the concentrate by flash chromatography on silica **gel** (ethyl acetate:hexane (1:3)+1% methanol) gave the title compound (320 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then methylene chloride:hexane:methanol (10:2:1)) to give the title compound (232 mg). (.sup.1 H NMR was consistent. . .

DETD . . . extracted from half-saturated sodium bicarbonate. The organic portion was dried over magnesium sulfate and purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol) to give the title compound (112 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . extracted with ethyl acetate (3.times.15 ml) and dried over magnesium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (2:1+1% methanol) to give the title compound (80.2 mg). (.sup.1 H NMR was consistent with the desired structure).

DETD . . . washed with a saturated brine solution and dried over sodium sulfate. The concentrate was purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:1)+1% methanol, then (4:1)+1% methanol) to give the title compound (680 mg).

DETD . . . over anhydrus sodium sulfate, filtered, and concentrated in vacuo. The product was isolated and purified by preparative tlc on silica **gel** (3:1, hexane/acetone) to give the desired product (105 mg).

DETD . . . washed with brine. The combined organics were dried over magnesium sulfate and the concentrate purified by preparative tlc on silica **gel** (2:1, hexane/acetone) to give the title compound (6 mg).

DETD . . . room temperature. After 1.5 hours, the solution was concentrated in vacuo and the mixture purified by preparative tlc on silica **gel** (2:1 hexane/acetone) to give the title compound (20 mg). MASS (FAB) 940 (M+Li).

DETD . . . room temperature. After 18 hours, the solvent was removed in vacuo and the mixture purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (62 mg). (.sup.1 H NMR consistent with the desired structure).

DETD . . . mL acetonitrile:hexane (3:1). The acetonitrile layer was dried over magnesium sulfate, and the concentrate purified by flash chromatography on silica **gel** (ethyl acetate:hexane (1:2)+1% methanol) to give the title compound (58.8 mg). (.sup.1 H NMR consistent with the desired structure).

DETD . . . (GIBO)). Cells were pelleted by centrifugation at 1500 rpm for 8 minutes. Contaminating red cells were removed by treating the **pellet** with ammonium chloride lysing buffer (GIBO)) for 2 minutes at 4.degree. C. Cold medium was added and cells were again. . .

L9 ANSWER 61 OF 68 USPATFULL
AB A method for the treatment of a cutaneous, ocular, or mucosal pathological condition which is associated with immune response in a human or other mammal, that includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.
AN 93:76520 USPATFULL
TI **Topical** application of spiperone or derivatives thereof for treatment of pathological conditions associated with immune responses
IN Sharpe, Richard J., Gloucester, MA, United States
Arndt, Kenneth A., Newton Centre, MA, United States
Galli, Stephen J., Winchester, MA, United States
PA Beth Israel Hospital Association, Boston, MA, United States (U.S. corporation)
PI US 5244902 19930914 <--
AI US 1992-831429 19920205 (7)
RLI Continuation-in-part of Ser. No. US 1990-494744, filed on 16 Mar 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-396523, filed on 21 Aug 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Schenkman, Leonard
LREP Kilpatrick & Cody
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 13 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 931
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI **Topical** application of spiperone or derivatives thereof for treatment of pathological conditions associated with immune responses
PI US 5244902 19930914 <--
AB . . . cutaneous, ocular, or mucosal pathological condition which is associated with immune response in a human or other mammal, that includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.
SUMM This invention is in the area of the **topical** treatment of cutaneous, ocular, and mucosal hypersensitivity and hyperproliferative conditions induced by or associated with an immune response, that includes. . .
SUMM . . . Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, lichen planus, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions.. . .
SUMM . . . agents with partial utility for treating some of the above conditions include psoralen plus ultraviolet A (PUVA), cyclosporin A, or **azathioprine**, but the risk-to-benefit ratios for these agents is

unfavorable for most of the conditions described above.

SUMM U.S. Pat. No. 4,874,766 assigned to Janssen Pharmaceutica N.V. discloses a method for promoting wound-healing by **topical** administration of a serotonin-antagonist compound, including spiperone and its derivatives. Wound healing is a reparative process by which several types. . .

SUMM It is an object of the present invention to present a method for the **topical** treatment of cutaneous, mucosal and ocular pathology associated with immune responses.

SUMM It is yet another object of the present invention to present a method for the **topical** treatment of cutaneous, mucosal, or ocular hypersensitivity and epithelial hyperproliferation.

SUMM It is yet another object of the invention to present a method for the **topical** treatment of cutaneous, mucosal or ocular scarring.

SUMM . . . ocular, or mucosal condition in a human or other mammal resulting from pathology associated with an immune response, that includes **topical** application of an effective amount of spiperone or a spiperone derivative or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.

SUMM . . . exhibits a strong immunosuppressive activity when applied topically. The parent spiperone is used herein as the model of an active **topical** immunosuppressant. Spiperone derivatives are measured against this model, and are considered to be immunosuppressants if they suppress the leukocyte infiltration. . .

SUMM . . . administered topically in a suitable carrier to effectively immunosuppress the patient at the site of application. Because the application is **topical**, i.e., local, immunosuppression is achieved without producing systemic effects, most notably, the significant neuroleptic effect that is associated with the. . .

SUMM Spiperone and its active derivatives are useful as **topical** agents in treating contact dermatitis, atopic dermatitis, eczematous dermatitis, psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, **aphthous** ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions. The novel. . .

DRWD . . . hypersensitivity reactions. These data (mean .+-.SEM) are from the same mice whose ear thickness measurements are presented in FIG. 5. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**p<0.01).

DRWD FIGS. 8a,b,c Effect of **topical** treatment with spiperone on leukocyte infiltration associated with oxazolone-induced contact hypersensitivity reactions. These date (mean .+-.SEM) are from the same. . . are presented in FIGS. 7a,b,c. Biopsies were performed 24 hours (a, b) or 46 hours (c) after application of oxazolone. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (II=p<0.01). In FIG. 8a, the slight. . .

DRWD FIG. 10 Effect of **topical** treatment with spiperone on leukocyte infiltration associated with DNFB-induced contact hypersensitivity reactions. These data (mean .+-.SEM) are from the same mice whose ear thickness measurements are presented in FIG. 9. **Topical** treatment with spiperone significantly diminished the reactions when compared to those in vehicle-treated mice (**p<0.01).

The

slight effect of treatment. . .

DETD Mammals, and specifically humans, suffering from pathogenic cutaneous, ocular, or mucosal immune responses can be treated by **topical** administration to the patient of an effective amount of the spiperone derivative or its salt in the presence of a. . .

DETD Solutions or suspensions for **topical** application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,. . .

DETD Suitable vehicles or carriers for **topical** application are known, and include lotions, suspensions, **ointments**, **creams**, **gels**, tinctures, sprays, powders, pastes, slow-release transdermal patches, aerosols for asthma, suppositories for application to rectal, vaginal, nasal or oral mucosa,. . .

DETD Thickening agents, emollients, and stabilizers can be used to prepare **topical** compositions. Examples of thickening agents include petrolatum, beeswax, xanthan gum, or polyethylene glycol, humectants such as sorbitol, emollients such as mineral oil, lanolin and its derivatives, or squalene. A number of solutions and **ointments** are commercially available, especially for ophthalmic and dermatologic applications.

DETD Natural or artificial flavorings or sweeteners can be added to enhance the taste of **topical** preparations applied for local effect to mucosal surfaces. Inert dyes or colors can be added, particularly in the case of. . .

DETD . . . potential irritancy or neuropharmacological effects of the composition. See, in general, Arndt, K. A., P. V. Mendenhall, "The Pharmacology of **Topical** Therapy", Dermatology in General Medicine, 1987; T. B. Fitzpatrick, A. Z. Eisen, K. Wolff, I. M. Freedberg and K. F.. . .

DETD Spiperone and spiperone derivatives are capable of suppressing the immune response in humans and other mammals on **topical** application. As such, the compounds, or therapeutic compositions thereof, are useful for the treatment of a myriad of immunological disorders. Pathogenic immune responses that can be treated by **topical** application of spiperone or spiperone derivatives include contact dermatitis, atopic dermatitis, eczematous dermatitis, drug eruptions, lichen planus, psoriasis, alopecia areata,. . . Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, cutaneous lupus erythematosus, scleroderma, allergic reactions secondary to arthropod bite reactions, **aphthous** ulcers, conjunctivitis, keratoconjunctivitis, iritis, asthma and allergic asthma, vaginitis, Crohn's disease, ulcerative colitis and proctitis. These compounds can also be. . .

DETD . . . ensues from the dry eye state. Spiperone or its active derivatives can be provided as an ophthalmic drop or ophthalmic **ointment** to humans or other mammals, including dogs and cats, in an effective amount in a suitable vehicle. This **topical** ophthalmic treatment can also serve to correct corneal and conjunctival disorders exacerbated by tear deficiency and KCS, such as corneal. .

DETD . . . the tissue swelling and the leukocyte infiltration associated with the elicitation phase of contact hypersensitivity to either oxazolone or dinitrofluorobenzene. **Topical** treatment with spiperone also suppressed the sensitization phase of contact sensitivity. However, mice treated topically with spiperone, unlike those treated. . .

DETD **Topical** Spiperone Treatment

DETD . . . 64% less tissue swelling and 70% less leukocyte infiltration at sites of hapten challenge than did show that treatment with **topical** spiperone can effectively inhibit the sensitization phase of cutaneous contact hypersensitivity.

DETD Effects of **Topical** Spiperone on Expression of Contact Hypersensitivity

DETD . . . skin) to both surfaces of the ears. The right ears of control mice were similarly treated, but with vehicle alone. **Topical** administration of a 4.0% suspension of spiperone in absolute ethanol, propylene glycol, and olive oil one hour after hapten challenge. . .

DETD Although **topical** application of spiperone was extremely effective in diminishing both the tissue swelling and the leukocyte infiltration associated with contact hypersensitivity. . .

DETD To evaluate the effect of **topical** treatment with spiperone on contact hypersensitivity reactions elicited with a different hapten, the effect of **topical** treatment with a 0.5% suspension of spiperone on the contact hypersensitivity reactions elicited with DNFB was examined. **Topical** treatment with spiperone significantly diminished the tissue swelling associated with reactions to DNFB (by 45%, FIG. 9) and had an. . .

DETD Mice were sensitized to oxazolone as described in Example 1. Three days later, slow release indomethacin **pellets** (0.05 mg, 3 week release) were implanted subcutaneously under light ether anesthesia. The dose of indomethacin delivered by these **pellets** has been previously shown to completely block prostaglandin synthesis in mice, by Jun, D. D., et al., J. Invest. Dermatol.. . .

DETD . . . and variations of the present invention relating to methods for the treatment of pathology associated with immune responses that includes **topical** administration of an effective amount of spiperone or a spiperone derivative will be obvious to those skilled in the art. . .

CLM What is claimed is:

. . . H.sub.4 --, 2-thienyl, or 4--X--C.sub.6 H.sub.4 CH.sub.2 --; or its pharmaceutically acceptable salt, in a pharmaceutically-acceptable diluent or carrier for **topical** application.

L9 ANSWER 62 OF 68 USPATFULL

AB This invention relates to the use of Ruthenium Red as an immunosuppressive agent to prevent or significantly reduce graft rejection in organ and bone marrow transplantation. Ruthenium Red can also be used as an immunosuppressant drug for T lymphocyte mediated autoimmune diseases. Furthermore, Ruthenium Red may be useful in alleviating psoriasis.

AN 93:69619 USPATFULL

TI Use of ruthenium red as immunosuppressive agents

IN Dwyer, Donard S., Lexington, MA, United States
Esenther, Kristin, Ashland, MA, United States

PA Procept, Inc., Cambridge, MA, United States (U.S. corporation)

PI US 5238689 19930824 <--

AI US 1992-817536 19920107 (7)

DT Utility

FS Granted

EXNAM Primary Examiner: Cintins, Marianne M.; Assistant Examiner: Cook, Rebecca
LREP Hamilton, Brook, Smith & Reynolds
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 345
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5238689 19930824 <--
DETD . . . compound may reduce epidermal hyperplasia and, at the same time, diminish any contribution by T cells to the disease process. **Topical** application of Ruthenium Red in a **cream** or **ointment** could deliver locally high concentrations of the drug without significant systemic exposure. This may be the ideal treatment modality for psoriasis and perhaps other inflammatory skin disease, such as **pemphigus vulgaris**.
DETD . . . dosage formulations containing a physiologically acceptable vehicle and optional adjuvants and preservatives. Suitable physiologically acceptable vehicles include saline, sterile water, **cream** or **ointments**.
DETD . . . boost the immunosuppressive effect. Compounds that can be co-administered include steroids (e.g. methyl prednisolone acetate) and known immunosuppressants such as **azathioprine**, 15-deoxyspergualin. Dosages of these drugs will also vary depending upon the condition and individual to be treated.
CLM What is claimed:
. . . of claim 1, further comprising administering the composition with an immunosuppressant selected from the group consisting of cyclosporin, rapamycin, FK-506, **azathioprine** and 15-deoxyspergualin.

L9 ANSWER 63 OF 68 USPATFULL
AB Interleukin 2 (IL 2; T-cell growth factor), produced with and without costimulation by Burkitt's lymphoma line Daudi, is highly purified approximately over 37,000-fold to apparent homogeneity from lymphocyte-conditioned medium derived from normal human blood cells by .(NH.sub.4).sub.2 SO.sub.4 -precipitation, ion-exchange chromatogr

This invention was made with support in part under Grants CA 08748, CA 22507, CA 25608, CA 20194, CA 21525, CA31525, P01-CA-20194, AI 18 321-01, CA 23766 and CA 33050 awarded by the National Cancer Institute, National Institute of Health, DHEW. The government has certain rights in this invention.
AN 90:38492 USPATFULL
TI Purified interleukin 2
IN Mertelsmann, Roland, 301 Millwood Rd., Chappaqua, NY, United States 10514
Welte, Karl, 504 E. 81st St., New York, NY, United States 10028
Venuta, Salvatore, Via Cilea 183, 80127 Napoli, Italy
PI US 4925919 19900515 <--
AI US 1988-205423 19880610 (7)
DCD 20051018
RLI Division of Ser. No. US 1984-603580, filed on 25 Apr 1984, now patented, Pat. No. US 4778879, issued on 18 Oct 1988 which is a continuation-in-part of Ser. No. US 1982-370223, filed on 20 Apr 1982, now abandoned

DT Utility
FS Granted
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Azpuru, C.
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 3219
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 4925919 19900515 <--
SUMM . . . line Daudi, is purified approximately 37,000-fold to apparent homogeneity from lymphocyte-conditioned medium by (NH.sub.4).sub.2 SO.sub.4 -precipitation, ion-exchange chromatography (diethylaminoethyl cellulose), **gel** filtration (AcA 44 Ultrogel), and hydrophobic chromatography, preferably on Blue Agarose and on Procion.RTM.-Red Agarose. IL2 can also be separated. . .
SUMM . . . 84
precipitate
III
DEAE cellu-
135 183,000
1,356 50 62
lose (DE 52)
IV
AcA 44 Ultro-
40 145,000
3,625 135 49
gel
V Blue Agarose
0.96 87,680
91,333 3,382 30
VI
Redrocion .RTM.
0.055.sup.++
55,229
1,004,164
37,191 19
Agarose*

.sup.+ The IL 2 activity in. . .
SUMM . . . IL 2 produced in the absence of Daudi cells has a molecular weight of about 26,000 daltons as measured by **gel** filtration and yields IL 2 having two molecular weights of about 16,000 and 17,000 daltons after denaturation as measured by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. IL 2 produced in the presence of Daudi cells (10.sup.6 /ml) shows a molecular weight of approximately 14,500 daltons as measured by both **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis.
SUMM . . . and was free of any contaminating proteins as judged by silver staining and by I.sup.125 exolabelling in sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. It is also pyrogen-free as tested in rabbits. In this test doses of purified IL 2 were used comparable. . .
SUMM The work of Mier et al. [J. Immunology (1982) 128:1122] uses preparative **gel** electrophoresis so differs from the invention detailed herein. No use of Sendai virus or Daudi cells is found in Mier. . .
SUMM . . . over an anion-exchange chromatographic column (diethylaminoethylSepharose). IL 2 activity eluted as a broad peak centered at approximately 0.07M NaCl. Subsequent **gel**

filtration with an Ultrogel AcA 54 column separated the IL 2 from most of the detectable proteins, and this sequence. . . specific activity over the IL 2 in the serum-free lymphocyte-conditioned medium. The IL 2-containing material was further purified using polyacrylamide **gel** electrophoresis containing sodium dodecyl sulfate. The IL 2 activity corresponded to a pair of protein bands present in the 13,000 molecular weight region in the sodium dodecyl sulfate **gel**. This procedure has been reported by Mier et al. (1982) Supra and Frank et al., (1981) J. Immunol. 127:2361, for. . .

SUMM The highly purified IL 2 obtained by us appears to be free of any contaminating proteins in sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis after staining with a silver nitrate method [Merril, C. R., et al. (1979) Proc. Nat'l. Acad. Sci. U.S.A. 76:4335),. . .

SUMM Up to three functionally active bands were detected in this preparation.

Elution of the materials from the sliced **gels** possessed high specific activity. We found that the molecular species of IL 2 are dependent on the experimental conditions used. . .

SUMM . . . proteins from desired proteinaceous material by anion exchange;

effecting separation by molecular weight of the IL 2-containing proteinaceous material by **gel** filtration; and separating IL 2, which is highly hydrophobic, from other lymphokines of about the same molecular weight via hydrophobic. . .

DRWD FIG. 2 concerns **gel** filtration of IL 2 on AcA 44 Ultrogel. DE 52-purified IL 2 was loaded on an AcA 44 Ultrogel column. . .

DRWD FIG. 5 illustrates a sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis profile of various steps of IL 2 purification ((a) molecular weight standards: phosphorylase b (MW 94,000), bovine serum albumin. . . ammonium sulfate precipitate; (d) pool of IL 2-containing diethylaminoethyl cellulose eluate; (e) IL 2-containing fractions pooled from AcA 44 Ultrogel **gel** filtration).

DRWD FIG. 6 shows the sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of Blue Agarose- and Procion.RTM.-Red Agarose-purified IL 2. IL 2 was treated with 2% sodium dodecyl sulfate and 5 mM 2-mercaptoethanol and applied to a 5-20% gradient **gel**. The protein bands were visualized by a silver nitrate method. The following marker proteins (200 ng each) were used: ovalbumin. . .

DRWD FIG. 7 shows the IL2 activity of 1 mm **gel** slices after sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of Procion.RTM.-Red Agarose-purified IL 2 produced in the presence or absence of Daudi cells. The IL 2 preparations were treated with 2% sodium dodecyl sulfate and 5 mM 2-mercaptoethanol and applied to a 15% polyacrylamide **gel**. After electrophoresis, the **gel** was sliced into 1 mm sections and proteins eluted with 0.3 ml phosphate-buffered saline (pH 7.2). The eluted material was. . .

DRWD FIG. 8 relates to the **gel** filtration chromatography of Blue Agarose-purified IL 2 on high performance liquid chromatography in the presence and absence of sodium dodecyl. . . native or treated with 1%

sodium dodecyl sulfate and 10 mM dithiothreitol, was applied to a high performance liquid chromatography **gel** filtration column. The following protein standards were used: bovine serum albumin (MW 68,000),

ovalbumin (MW 43,000), chymotrypsinogen (MW 25,000), and. . .

DRWD . . . represents the 100% range of normal donors (n=21) in the presence or absence of highly purified IL-2. O*, *=patients with **GvHD**. --=median .sup.3 H-thymidine uptake.

DRWD . . . represents the 100% range of normal donors (n=21) in the

presence or absence of highly purified IL-2. O*, *=patients with **GvHD**. --=median .sup.3 H-thymidine uptake.

DRWD . . . represents the 100% range of normal donors (n=21) in the presence or absence of highly purified IL-2. O*, *=patients with **GvHD**. --=median .sup.3 H-thymidine uptake.

DETD . . . dialyzed ammonium sulfate precipitate. After 30 minutes the diethylaminoethyl cellulose was spun down and the supernatant saved (Supernatant 1). The **pellet** was resuspended in 300 ml of 0.05M Tris-HCl (pH 7.8) containing 0.01M NaCl. After 10 minutes the diethylaminoethyl cellulose was. . .

DETD **Gel** Filtration (Fraction IV)

DETD . . . al. (1951) J. Biol. Chem. 193:265]. For protein concentrations lower than 5 micrograms/ml, samples were subjected to sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis; the protein bands were visualized by the silver staining technique [Merril, C. R., (1979) Supra]; and the protein concentration. . .

DETD Sodium Dodecyl Sulfate-Polyacrylamide **Gel** Electrophoresis

DETD The discontinuous Tris-glycine system of Laemmli [Laemmli, U. K., (1970)

227:680] was used for 1.5-mm thick slab **gels** using a 5-20% gradient or a 15% of acrylamide. The samples were analyzed under both reduced (2% sodium dodecyl sulfate, 5% mercaptoethanol) and non-reduced (2% sodium dodecyl sulfate) conditions. After electrophoresis, **gels** were stained with Coomassie Brilliant Blue or by a silver nitrate method [Merril, C. R., et al., (1979) Supra]. Apparent. . . 68,000), ovalbumin (MW 43,000), carbonic anhydrase (MW 30,000), soybean trypsin inhibitor (MW 20,000) and alpha-lactalbumin (MW 14,500). After electrophoresis, the **gels** were sliced into 1-mm sections and proteins from each slice were eluted in 0.3 ml phosphate-buffered saline

(pH 7.2). After. . .

DETD . . . to O'Farrell [(1975) J. Biol. Chem. 250:4007], with 3/10 Biolyte (Bio-Rad) in the first dimension and a 10% acrylamide uniform **gel** in the second dimension. Isoelectric focusing was at 500 V for 20 hr; slab **gels** were run at 20 mA/**gel**.

DETD Staining: To stain the **gels** with silver (2) they are fixed in 50% methanol/12% acetic acid for 30 min (**gels** can be stored overnight in this solution). The **gels** tend to shrink in the 50% methanol solution. To expand them prior to staining, they are placed

in 10% ethanol/5% acetic acid for 2 hr, followed by three washes with 10% ethanol (5 min each). The **gels** are then soaked in 4% (wt/vol) paraformaldehyde/1.43% (wt/vol) sodium cacodylate (adjusted to pH 7.3 with HCl) for 30 min., followed by three 5-min washes with 10% ethanol. The **gels** are then agitated gently for 30 min in a cupric nitrate/silver nitrate solution (made by dissolving 3.5 g of silver. . .

DETD Next the **gels** are placed in fresh diammine solution (made within 5 min of use) prepared by mixing together 30 ml of a. . . remaining after the procedure must be discarded because an explosive complex may form upon storage!. After the diammine rinse, the **gels** were washed for 1 min in a reducing solution containing 2.5 ml of 10% formaldehyde (10 ml of commercial formaldehyde. . . appear as brown or black spots at any time in the reducing solutions. Staining can be stopped by washing the **gel** in successive changes of deionized water. Image formation in the diammine step may occur if reagent-grade absolute ethanol and fresh. . . washing the glass slab plates thoroughly, immediately after each electrophoresis run, and using

well washed surgical gloves when handling the **gels**. The

gels are fragile after staining and should be photographed for a permanent record.

DETD **Gels** that are overdeveloped may be lightened with a photographic reducer such as the copper reducer of Smith [Walls, E. J.,.

. . sodium thiosulfate in 1 liter of water. Usually a 3:1 dilution cf water to fresh reducer is used to lighten **gels**. The reduction is stopped by washing the **gel** in water.

DETD Stained **gels** may be kept in water. **Gels** that are to be dried for storage or autoradiography should be first soaked in 30% (wt/vol) sodium thiosulfate for 15 min followed by four 15-min water rinses. The **gels** should then be soaked for 5 min in a preserving solution [methanol/H.sub.2 O/glycerol, 70:27:3 (vol/vol) [Mayer J. W., (1976) Anal.. . .

DETD Autoradiography: **Gels** that were to be autoradiographed were dried as described above and then placed in x-ray film cassette holders (Kodak X-omatic,. . .

DETD . . . requires centrifugation to remove the Enzymobead Reagent from the reaction mixture, followed by immediate removal of the supernatant for subsequent **gel** filtration. The second method used for IL2 utilizes direct application of the test tube mixture to a **gel** filtration column. In both cases, a Bio-**Gel** P-6 DG column is recommended for the separation of the unbound iodide from the labeled protein.

---

*Since multiple isotopic substitution of a. . .

DETD . . . concentrations, as shown in FIG. 4. The 0.7M-0.8M NaCl eluate pool was found, through silver staining of a 5-20% gradient **gel** (sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis), to contain three molecular components with molecular weights of 14,500.+-.2000, 16,000+1000 and 17,000.+-.1000 daltons depending on the experimental condition. . .

DETD The IL 2 preparation from various steps of purification were subjected to sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis analysis. Preparations obtained prior to the Blue Agarose chromatography (Fractions I-IV) were analyzed on a 5-20% gradient **gel** followed by Coomassie brillant blue staining as shown in FIG. 5. Preparations obtained after Blue Agarose chromatography and Procion.RTM.-Red Agarose chromatography were also analyzed on a 5-20% gradient **gel** followed by the highly sensitive silver staining method as shown in FIG. 6.

DETD To obtain a better resolution, the purified IL 2 was also analyzed on a 15% acrylamide **gel**. After staining, a molecular weight pattern similar to that obtained in the gradient **gel** was found. A parallel **gel** was sliced into 1-mm sections and proteins from each slice were eluted in phosphate-buffered saline (pH 7.2). Il 2 activity. . .

DETD . . . dodecyl sulfate and 20 mM dithiothreitol at 37.degree. C. for 1 hour and applied to an high performance liquid chromatography **gel** filtration column. The column was eluted with buffer containing 0.1% sodium dodecyl sulfate and 1 mm dithiothreitol. As shown in. . .

DETD . . . which contaminate most partially purified IL 2 preparations. For example, alpha-Interferon co-purified with IL 2 during ion exchange chromatography and **gel** filtration steps, but was clearly

separated from IL 2 by Blue Agarose chromatography. See FIG. 3. After chromatography on Procion.RTM.-Red. . .

DETD . . . the 26,000-dalton IL 2 of the invention exhibited a molecular weight of 16,000-18,000 daltons by high performance liquid filtration chromotography **gel**. Sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of this denatured form demonstrated the presence of two biologically active bands with molecular weights of about 16,000 and. . .

DETD . . . a specific activity of 10.sup.6 U/mg of protein, and consists of two active bands on a silver-stained sodium dodecyl sulfate-polyacrylamide **gel** [Welte, K, et al. (1982) Supra].

DETD . . . patients received only preparative chemotherapy with cyclophosphamide (50 mg/kg for 4 days), cytosine arabinoside (200 mg/kg/day for 5 days) and 6-**thioguanine** (200 mg/kg/day for 5 days), whereas 2 other patients were conditioned similar to patients with leukemias but with less TBI. . . and weekly thereafter to day 100. All immune suppressive drugs were stopped at that time. Patients with graft- versus-host disease (**GvHD**) of at least grade 2 were treated with high dose prednisone (2 mg/kg/d). One AML patient received prednisone plus cyclosporine A (10 mg/kg/d) while another ALL patient was maintained only on **azathioprine** (50 mg/d). At the time of the IL2 analysis, 13 patients had **GvHD** (4 patients grade 1;3 patients grade 2; and 6 patients grade 3). Four of the 13 patients had acute **GvHD**.

DETD . . . production or proliferative responses to OKT3 or PHA, respectively, in the absence or presence of exogenous hpIL2. The Effect of **GvHD** and Immunosuppressive Drugs on Mitogen Responses to OKT3 Antibody:

DETD . . . given no further immunosuppressive therapy after BMT. All patients receiving allogeneic BMT, were treated with prophylactic methotrexate, while those with **GvHD** received, in addition, high dose prednisone (see above). Only one patient received prednisone plus cyclosporine A for **GvHD**. No differences between groups were seen with respect to endogenous IL2 production (Table XIV) and proliferative responses to OKT3 antibody. . .

DETD The study group included 13 patients who developed acute or chronic **GvHD** (grade 1-3) (shown with asterisks beside the symbols in FIG. 1). There were no statistically significant differences in the mitogen responses nor in the restoration of proliferation of PBMC by hpIL2 between patients with or without **GvHD**.

DETD . . . to PHA. IL2 has previously been shown to be able to restore (a) impaired cell-mediated lympholysis in patients with acute **GvHD** but not chronic **GvHD** [Mori, T, et al.(1983) J. Immunol. 130:712] and (b) PHA stimulated T cell colony-formation of lymphocytes from patients early after. . .

DETD . . . Molecular and Cellular Biology, Steamboat Springs 1983 (in press)]. However, in patients after BMT IL2 might enhance or cause acute **GvHD**. Animal studies have been initiated to address this problem.

CLM What is claimed is:

. . . Purified human interleukin-2 having apparent homogeneity and characterized by: (a) a molecular weight of about 14,500.+-.2,000 daltons as measured by **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis; and (b) a specific activity of at least 9.times.10.sup.5 U/mg in the murine interleukin-2 dependent cytotoxic T-cell line assay.

. . .

4. A purified human interleukin-2 of claim 1 having no contaminating

proteins as determined by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis and staining with silver nitrate or exolabelling with I.sup.125.

. . . Purified human interleukin-2 having apparent homogeneity and characterized by: (a) a molecular weight of about 26,000.+-.4,000 daltons as measured by **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis; and a specific activity of at least 9.times.10.sup.5 U/mg in the murine interleukin-2 dependent cytotoxic T-cell line assay.

10. A purified human interleukin-2 of claim 7 having no contaminating proteins as determined by sodium dodecy-1 sulfate-polyacrylamide **gel** electrophoresis and staining with silver nitrate or exolabelling with I.sup.125.

. . . Purified human interleukin-2 having apparent homogeneity and characterized by: (a) a molecular weight of about 16,000.+-.1,000 daltons as measured by **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis; and (b) a specific activity of at least 9.times.10.sup.5 U/mg in the murine interleukin-2 dependent cytotoxic T-cell line assay.

. . .

16. A purified human interleukin-2 of claim 13 having no contaminating proteins as determined by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis and staining with silver nitrate or exolabelling with I.sup.125.

. . . 18. Purified human interleukin-2 having apparent homogeneity and characterized by: a molecular weight of about 17,000.+-.1,000 daltons as

measured by **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis; and a specific activity of at least 9.times.105 U/mg in the murine interleukin-2 dependent cytotoxic T-cell line assay.

21. A purified human interleukin-2 of claim 18 having no contaminating proteins as determined by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis and staining with silver nitrate or exolabelling with I.sup.125.

L9 ANSWER 64 OF 68 USPATFULL

AB Although fenclofenac (2-(2,4-dichloro-phenoxy) phenyl acetic acid) is known as an NSAID it has now been shown to have immunosuppressive properties indicating its usefulness in the treatment of a wide variety of conditions requiring immunosuppressive therapy. In this role fenclofenac may be combined with a prostaglandin and/or another immunosuppressive drug, and may be administered in a form for release in

the terminal ileum or colon.

AN 90:23636 USPATFULL

TI Uses of a substituted 2-phenoxyphenylacetic acid as an immunosuppressant

drug

IN Wood, Elizabeth M., Lubnaig, 442 Blackness Road, Dundee, United Kingdom DD2 1TQ

PI US 4912136 19900327 <--

AI US 1988-212915 19880629 (7)

PRAI GB 1987-15242 19870629

DT Utility
FS Granted
EXNAM Primary Examiner: Friedman, Stanley J.
LREP Reynolds, Florence U.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 16 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 581
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 4912136 19900327 <--
SUMM . . . disease
Polymyositis
Dermatomyositis
Diseases of blood vessels
Vasculitis
Polyarteritis nodosa
Auto-immune haematological disorders
Inflammatory bowel disease
Crohn's disease
Ulcerative colitis
Coeliac disease
Chronic active hepatitis
Neurological diseases
Myasthenia gravis
Multiple sclerosis
Guillain Barre syndrome
Skin diseases
Pemphigus
Bullous **pemphigoid**
Dermatitis herpetiformis
Psoriasis
Auto-immune endocrine diseases
1. Type I Diabetes
(Juvenile type or insulin dependent)
2. Auto-immune thyroid diseases
Hashimoto's thyroiditis
Atrophic hypothyroidism
Grave's disease
Grave's. . .
SUMM Although in severe and acute phases of all the above-described disease processes, immunosuppression with conventional drugs such as steroids, **azathioprine** and cyclosporin may be required, for chronic use and for milder cases, fenclofenac may be a suitable drug. Fenclofenac may. . .
SUMM . . . are unable to take oral preparations, parental preparations of fenclofenac with pharmaceutically inactive diluents or carriers may be used. A **topical** preparation of fenclofenac may also be used in skin diseases e.g. psoriasis, the preparation comprising fenclofenac and
a suitable carrier, for example ethyl alcohol, or a conventional lotion or **cream** base.

L9 ANSWER 65 OF 68 USPATFULL
AB Interleukin 2 (IL 2; T-cell growth factor), produced with and without costimulation by Burkitt's lymphoma line Daudi, is highly purified approximately over 37,000-fold to apparent homogeneity from lymphocyte-conditioned medium derived from normal huma

This invention was made with support in part under Grants CA 08748, CA 22507, CA 25608, CA 20194, CA 21525, CA 31525, P01-CA-20194, AI 18

321-01, CA 23766 and CA 33050 awarded by the National Cancer Institute, National Institute of Health, DHEW. The government has certain rights in this invention.

AN 90:19637 USPATFULL

TI Process for preparing purified interleukin-2

IN Mertelsmann, Roland, Chappagua, NY, United States
Welte, Karl, New York, NY, United States
Venuta, Salvatore, Napoli, Italy

PA Sloan-Kettering institute for Cancer Research, New York, NY, United States (U.S. corporation)

PI US 4908434 19900313 <--

AI US 1988-205172 19880610 (7)

RLI Division of Ser. No. US 1984-603580, filed on 25 Apr 1984, now patented, Pat. No. US 4778879, issued on 18 Oct 1988 which is a continuation-in-part of Ser. No. US 1982-370223, filed on 20 Apr 1982, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Kight, John; Assistant Examiner: Azpuru, C.

LREP White, John P.

CLMN Number of Claims: 16

ECL Exemplary Claim: 1

DRWN 12 Drawing Figure(s); 11 Drawing Page(s)

LN.CNT 3219

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 4908434 19900313 <--

SUMM . . . line Daudi, is purified approximately 37,000-fold to apparent homogeneity from lymphocyte-conditioned medium by (NH.sub.4).sub.2 SO.sub.4 -precipitation, ion-exchange chromatography (diethylaminoethyl cellulose), **gel** filtration (AcA 44 Ultrogel), and hydrophobic chromatography, preferably on Blue Agarose and on Procion.RTM.-Red Agarose. IL2 can also be separated. . .

SUMM

				. . . 9,000	247,000
		27		84	
precipitate					
III DEAE cellu-					
	135	183,000			
		1,356	50	62	
lose (DE 52)					
IV AcA 44 Ultro-					
	40	145,000			
		3,625	135	49	
gel					
V Blue Agarose					
	0.96	87,680			
		91,333	3,382	30	
RedProcion .RTM.					
	0.055.sup.++				
		55,229			
		1,004,164			
			37,191		
				19	
Agarose*					

.sup.+ The IL 2 activity in the. . .

SUMM . . . IL 2 produced in the absence of Daudi cells has a molecular weight of about 26,000 daltons as measured by **gel** filtration and yields IL 2 having two molecular weights of about 16,000 and 17,000

daltons after denaturation as measured by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. IL 2 produced in the presence of Daudi cells (10.sup.6 /ml) shows a molecular weight of approximately 14,500 daltons as measured by both **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis.

SUMM . . . and was free of any contaminating proteins as judged by silver staining and by I.sup.125 exolabelling in sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. It is also pyrogen-free as tested in rabbits. In this test doses of purified IL 2 were used comparable. . .

SUMM The work of Mier et al. [J. Immunology (1982) 128:1122] uses preparative **gel** electrophoresis so differs from the invention detailed herein. No use of Sendai virus or Daudi cells is found in Mier. . .

SUMM . . . over an anion-exchange chromatographic column (diethylaminoethyl-Sepharose). IL 2 activity eluted as a broad peak centered at approximately 0.07M NaCl. Subsequent **gel** filtration with an Ultrogel AcA 54 column separated the IL 2 from most of the detectable proteins, and this sequence. . . specific activity over the IL 2 in the serum-free lymphocyte-conditioned medium. The IL 2-containing material was further purified using polyacrylamide **gel** electrophoresis containing sodium dodecyl sulfate. The IL 2 activity corresponded to a pair of protein bands present in the 13,000 molecular weight region in the sodium dodecyl sulfate **gel**. This procedure has been reported by Mier et al. (1982) Supra and Frank et al., (1981) J. Immunol. 127:2361, for. . .

SUMM . . . The highly purified IL 2 obtained by us appears to be free of any contaminating proteins in sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis after staining with a silver nitrate method [Merril, C. R., et al. (1979) Proc. Nat'l. Acad. Sci. U.S.A. 76:4335],. . .

SUMM Up to three functionally active bands were detected in this preparation. Elution of the materials from the sliced **gels** possessed high specific activity. We found that the molecular species of IL 2 are dependent on the experimental conditions used. . .

SUMM . . . proteins from desired proteinaceous material by anion exchange; effecting separation by molecular weight of the IL 2-containing proteinaceous material by **gel** filtration; and separating IL 2, which is highly hydrophobic, from other lymphokines of about the same molecular weight via hydrophobic. . .

DRWD FIG. 2 concerns **gel** filtration of IL 2 on AcA 44 Ultrogel. DE 52-purified IL 2 was loaded on an AcA 44 Ultrogel column. . .

DRWD FIG. 5 illustrates a sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis profile of various steps of IL 2 purification ((a) molecular weight standards: phosphorylase b (MW 94,000), bovine serum albumin. . . ammonium sulfate precipitate; (d) pool of IL 2-containing diethylaminoethyl cellulose eluate; (e) IL 2-containing fractions pooled from AcA 44 Ultrogel **gel** filtration).

DRWD FIG. 6 shows the sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of Blue Agarose- and Procion.RTM.-Red Agarose-purified IL 2. IL 2 was treated with 2% sodium dodecyl sulfate and 5 mM 2-mercaptoethanol and applied to a 5-20% gradient **gel**. The protein bands were visualized by a silver nitrate method. The following marker proteins (200 ng each) were used: ovalbumin. . .

DRWD FIG. 7 shows the IL 2 activity of 1 mm **gel** slices after sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of Procion.RTM.-Red Agarose-purified IL 2 produced in the presence or

absence of Daudi cells. The IL 2 preparations were treated with 2% sodium dodecyl sulfate and 5 mM 2-mercaptoethanol and applied to a 15% polyacrylamide **gel**. After electrophoresis, the **gel** was sliced into 1 mm sections and proteins eluted with 0.3 ml phosphate-buffered saline (pH 7.2). The eluted material was. . .

DRWD FIG. 8 relates to the **gel** filtration chromatography of Blue Agarose-purified IL 2 on high performance liquid chromatography in the presence and absence of sodium dodecyl. . . native or treated with 1% sodium dodecyl sulfate and 10 mM dithiothreitol, was applied to a high performance liquid chromatography **gel** filtration column. The following protein standards were used: bovine serum albumin (MW 68,000), ovalbumin (MW 43,000), chymotrypsinogen (MW 25,000), and. . .

DETD . . . dialyzed ammonium sulfate precipitate. After 30 minutes the diethylaminoethyl cellulose was spun down and the supernatant saved (Supernatant 1). The **pellet** was resuspended in 300 ml of 0.05M Tris-HCl (pH 7.8) containing 0.01M NaCl. After 10 minutes the diethylaminoethyl cellulose was. . .

DETD **Gel** Filtration (Fraction IV)

DETD . . . al. (1951) J. Biol. Chem. 193:265]. For protein concentrations lower than 5 micrograms/ml, samples were subjected to sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis; the protein bands were visualized by the silver staining technique [Merril, C. R., (1979) Supra]; and the protein concentration. . .

DETD Sodium Dodecyl Sulfate-Polyacrylamide **Gel** Electrophoresis

DETD The discontinuous Tris-glycine system of Laemmli [Laemmli, U. K., (1970) 227:680] was used for 1.5-mm thick slab **gels** using a 5-20% gradient or a 15% of acrylamide. The samples were analyzed under both reduced (2% sodium dodecyl sulfate, 5% mercaptoethanol) and non-reduced (2% sodium dodecyl sulfate) conditions. After electrophoresis, **gels** were stained with Coomassie Brilliant Blue or by a silver nitrate method [Merril, C. R., et al., (1979) Supra]. Apparent. . . 68,000), ovalbumin (MW 43,000), carbonic anhydrase (MW 30,000), soybean trypsin inhibitor (MW 20,000) and alpha-lactalbumin (MW 14,500). After electrophoresis, the **gels** were sliced into 1-mm sections and proteins from each slice were eluted in 0.3 ml phosphate-buffered saline (pH 7.2). After. . .

DETD . . . to O'Farrell [(1975) J. Biol. Chem. 250:4007], with 3/10 Biolyte (Bio-Rad) in the first dimension and a 10% acrylamide uniform **gel** in the second dimension. Isoelectric focusing was at 500 V for 20 hr; slab **gels** were run at 20 mA/**gel**.

DETD Staining: To stain the **gels** with silver (2) they are fixed in 50% methanol/12% acetic acid for 30 min (**gels** can be stored overnight in this solution). The **gels** tend to shrink in the 50% methanol solution. To expand them prior to staining, they are placed in 10% ethanol/5% acetic acid for 2 hr, followed by three washes with 10% ethanol (5 min each). The **gels** are then soaked in 4% (wt/vol) paraformaldehyde/1.43% (wt/vol) sodium cacodylate (adjusted to pH 7.3 with HCl) for 30 min., followed by three 5-min washes with 10% ethanol. The **gels** are then agitated gently for 30 min in a cupric nitrate/silver nitrate solution (made by dissolving 3.5 g of silver. . .

DETD Next the **gels** are placed in fresh diammine solution (made within 5 min of use) prepared by mixing together 30 ml of a. . . remaining after the procedure must be discarded because an explosive complex may form upon storage! After the diammine rinse, the

gels were washed for 1 min in a reducing solution containing 2.5 ml of 10% formaldehyde (10 ml of commercial formaldehyde. . . appear as brown or black spots at any time in the reducing solutions. Staining can be stopped by washing the **gel** in successive changes of deionized water. Image formation in the diammine step may occur if reagent-grade absolute ethanol and fresh. . . washing the glass slab plates thoroughly, immediately after each electrophoresis run, and using

well washed surgical gloves when handling the **gels**. The **gels** are fragile after staining and should be photographed for a permanent record.

DETD **Gels** that are overdeveloped may be lightened with a photographic reducer such as the copper reducer of Smith [Walls, E. J.,.

. . sodium thiosulfate in 1 liter of water. Usually a 3:1 dilution of water to fresh reducer is used to lighten **gels**. The reduction is stopped by washing the **gel** in water.

DETD Stained **gels** may be kept in water. **Gels** that are to be dried for storage or autoradiography should be first soaked in 30% (wt/vol) sodium thiosulfate for 15 min followed by four 15-min water rinses. The **gels** should then be soaked for 5 min in a preserving solution [methanol/H.sub.2 O/glycerol, 70:27:3 (vol/vol) [Mayer J. W., (1976) Anal.. . .

DETD Autoradiography: **Gels** that were to be autoradiographed were dried as described above and then placed in x-ray film cassette holders (Kodak X-omatic,. . .

DETD . . . requires centrifugation to remove the Enzymobead Reagent from the reaction mixture, followed by immediate removal of the supernatant for subsequent **gel** filtration. The second method used for IL 2 utilizes direct application of the test tube mixture to a **gel** filtration column.

DETD In both cases, a Bio-**Gel** P-6 DG column is recommended for the separation of the unbound iodide from the labeled protein.

DETD . . . concentrations, as shown in FIG. 4. The 0.7M-0.8M NaCl eluate pool was found, through silver staining of a 5-20% gradient **gel** (sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis), to contain three molecular components with molecular weights of 14,500 .+-.2000, 16,000+1000 and 17,000.+-.1000 daltons depending on the experimental. . .

DETD The IL 2 preparation from various steps of purification were subjected to sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis analysis. Preparations obtained prior to the Blue Agarose chromatography

(Fractions I-IV) were analyzed on a 5-20% gradient **gel** followed by Coomassie brilliant blue staining as shown in FIG. 5. Preparations obtained after Blue Agarose chromatography and Procion.RTM.-Red Agarose chromatography were also analyzed on a 5-20% gradient **gel** followed by the highly sensitive silver staining method as shown in FIG. 6.

DETD To obtain a better resolution, the purified IL 2 was also analyzed on a 15% acrylamide **gel**. After staining, a molecular weight pattern similar to that obtained in the gradient **gel** was found. A parallel **gel** was sliced into 1-mm sections and proteins from each slice were eluted in phosphate-buffered saline (pH 7.2). Il 2 activity. . .

DETD . . . dodecyl sulfate and 20 mM dithiothreitol at 37.degree. C. for 1

hour and applied to an high performance liquid chromatography **gel** filtration column. The column was eluted with buffer containing 0.1% sodium dodecyl sulfate and 1 mM dithiothreitol. As shown

in. . .

DETD . . . factors which contaminate most partially purified IL 2 preparations. For example, alpha-Interferon co-purified IL 2 during ion exchange chromatography and **gel** filtration steps, but was clearly separated from IL 2 by Blue Agarose chromatography. See FIG. 3. After chromatography on Procion.RTM.-Red. . .

DETD . . . sulfate, the 26,000-dalton IL 2 of the invention exhibited a molecular weight of 16,000-18,000 daltons by high performance liquid chromatography **gel** filtration. Sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of this denatured form demonstrated the presence of two biologically active bands with molecular weights of about 16,000 and. . .

DETD . . . a specific activity of 10.sup.6 U/mg of protein, and consists of two active bands on a silver-stained sodium dodecyl sulfate-polyacrylamide **gel** [Welte, K, et al. (1982) Supra].

DETD . . . patients received only preparative chemotherapy with cyclophosphamide (50 mg/kg for 4 days), cytosine arabinoside (200 mg/kg/day for 5 days) and 6-**thioguanine** (200 mg/kg/day for 5 days), whereas 2 other patients were conditioned similar to patients with leukemias but with less TBI. . . and weekly thereafter to day 100. All immune suppressive drugs were stopped at that time. Patients with graft- versus-host disease (**GvHD**) of at least grade 2 were treated with high dose prednisone (2 mg/kg/d). One AML patient received prednisone plus cyclosporine A (10 mg/kg/d) while another ALL patient was maintained only on **azathioprine** (50 mg/d). At the time of the IL2 analysis, 13 patients had **GvHD** (4 patients grade 1;3 patients grade 2; and 6 patients grade 3). Four of the 13 patients had acute **GvHD**.

DETD The Effect of **GvHD** and Immunosuppressive Drugs on Mitogen Responses to OKT3 Antibody: The study population consisted of three groups with respect to immunosuppressive. . . given no further immunosuppressive therapy after BMT. All patients receiving allogeneic BMT, were treated with prophylactic methotrexate, while those with **GvHD** received, in addition, high dose prednisone (see above) Only one patient received prednisone plus cyclosporine A for **GvHD**. No differences between groups were seen with respect to endogenous IL2 production (Table XIV) and proliferative responses to OKT3 antibody. . .

DETD The study group included 13 patients who developed acute or chronic **GvHD** (grade 1-3) (shown with asterisks beside the symbols in FIG. 1). There were no statistically significant differences in the mitogen responses nor in the restoration of proliferation of PBMC by hpIL2 between patients with or without **GvHD**.

DETD . . . to PHA. IL2 has previously been shown to be able to restore (a) impaired cell-mediated lympholysis in patients with acute **GvHD** but not chronic **GvHD** [Mori, T., et al. (1983) J. Immunol. 130:712] and (b) PHA stimulated T cell colony-formation of lymphocytes from patients early. . .

DETD . . . Molecular and Cellular Biology, Steamboat Springs 1983 (in press)]. However, in patients after BMT IL2 might enhance or cause acute **GvHD**. Animal studies have been initiated to address this problem.

CLM What is claimed is:

. . . separate undesired, non-specific proteinaceous material from the human interleukin-2-containing proteinaceous components; (d) subjecting the resulting human interleukin-2-containing proteinaceous material to **gel** filtration chromatography so as to recover the human interleukin-2 and other proteinaceous material of about the same

molecular weight; and. . .

L9 ANSWER 66 OF 68 USPATFULL
AB Interleukin 2 (IL 2; T-cell growth factor), produced with and without costimulation by Burkitt's lympho

This invention was made with support in part under Grants CA 08748, CA 22507, CA 25608, CA 20194, CA 21525, CA31525, PO1-CA-20194, AI 18 321-01, CA 23766 and CA 33050 awarded by the National Cancer Institute, National Institute of Health, DHEW. The government has certain rights in this invention.
AN 90:19636 USPATFULL
TI Uses of interleukin-2
IN Mertelsmann, Roland, Chappaqua, NY, United States
Welte, Karl, New York, NY, United States
Venuta, Salvatore, Napoli, Italy
PA Sloan-Kettering Institute for Cancer Research, New York, NY, United States (U.S. corporation)
PI US 4908433 19900313 <--
AI US 1988-205451 19880610 (7)
RLI Division of Ser. No. US 1984-603580, filed on 25 Apr 1984, now patented, Pat. No. US 4778879, issued on 18 Oct 1988 which is a continuation-in-part of Ser. No. US 1982-370223, filed on 20 Apr 1982, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Azpuru, Carlos
LREP White, John P.
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 3205
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 4908433 19900313 <--
SUMM . . . line Daudi, is purified approximately 37,000-fold to apparent homogeneity from lymphocyte-conditioned medium by (NH.sub.4).sub.2 SO.sub.4 -precipitation, ion-exchange chromatography (diethylaminoethyl cellulose), **gel** filtration (AcA 44 Ultrogel), and hydrophobic chromatography, preferably on Blue Agarose and on Procion.sup.R -Red Agarose. IL2 can also be. . .
SUMM . . . -

	9,000	247,000	27		84
precipitate					
III DDAE cellu-					
	135	183,000	1,356	50	62
lose(DE 52)					
IV AcA 44 Ultro-					
	40	145,000	3,625	135	49
gel					
V Blue Agarose					
	0.96	87,680	91,333	3,382	30
VI Procion.sup.R -Red					
	0.055.sup.++				
		55,229	1,004,164		
			37,191		
				19	
Agarose*					

.sup.+ The IL 2 activity in. . .

SUMM . . . IL 2 produced in the absence of Daudi cells has a molecular weight of about 26,000 daltons as measured by **gel** filtration and yields IL 2 having two molecular weights of about 16,000 and 17,000 daltons after denaturation as measured by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. IL 2 in the presence of Daudi cells (10.sup.6 /ml) shows a molecular weight of approximately 14,500 daltons as measured by both **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis.

SUMM . . . and was free of any contaminating proteins as judged by silver staining and by I.sup.125 exolabelling in sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. It is also pyrogen-free as tested in rabbits. In this test doses of purified IL 2 were used comparable. . .

SUMM The work of Mier et al. [J. Immunology (1982) 128:1122] uses preparative **gel** electrophoresis so differs from the invention detailed herein. No use of Sendai virus or Daudi cells is found in Mier. . .

SUMM . . . over an anion-exchange chromatographic column (diethylaminoethyl-Sepharose). IL 2 activity eluted as a broad peak centered at approximately 0.07M NaCl. Subsequent **gel** filtration with an Ultrogel AcA 54 column separated the IL 2 from most of the detectable proteins, and this sequence. . . specific activity over the IL 2 in the serum-free lymphocyte-conditioned medium. The IL 2-containing material was further purified using polyacrylamide **gel** electrophoresis containing sodium dodecyl sulfate. The IL 2 activity corresponded to a pair of protein bands present in the 13,000 molecular weight region in the sodium dodecyl sulfate **gel**. This procedure has been reported by Mier et al. (1982) Supra and Frank et al., (1981) J. Immunol. 127:2361, for. . .

SUMM The highly purified IL 2 obtained by us appears to be free of any contaminating proteins in sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis after staining with a silver nitrate method [Merril, C. R., et al. (1979) Proc. Nat'l. Acad. Sci. U.S.A. 76:4335],. . .

SUMM Up to three functionally active bands were detected in this preparation. Elution of the materials from the sliced **gels** possessed high specific activity. We found that the molecular species of IL 2 are dependent on the experimental conditions used. . .

SUMM . . . proteins from desired proteinaceous material by anion exchange; effecting separation by molecular weight of the IL 2-containing proteinaceous material by **gel** filtration; and separating IL 2, which is highly hydrophobic, from other lymphokines of about the same molecular weight via hydrophobic. . .

DRWD FIG. 2 concerns **gel** filtration of IL 2 on AcA 44 Ultrogel. DE 52-purified IL 2 was loaded on an AcA 44 Ultrogel column. . .

DRWD FIG. 5 illustrates a sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis profile of various steps of IL 2 purification ((a) molecular weight standards: phosphorylase b (MW 94,000), bovine serum albumin. . . ammonium sulfate precipitate; (d) pool of IL 2-containing diethylaminoethyl cellulose eluate; (e) IL 2-containing fractions pooled from AcA 44 Ultrogel **gel** filtration).

DRWD FIG. 6 shows the sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of Blue Agarose-and Procion.sup.R -Red Agarose-purified IL 2. IL 2 was treated with 2% sodium dodecyl sulfate and 5 mM 2-mercaptoethanol and applied to a 5-20% gradient **gel**. The protein bands were visualized by a silver nitrate method. The following

marker proteins (200 ng each) were used: ovalbumin. . .

DRWD FIG. 7 shows the IL 2 activity of 1 mm **gel** slices after sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of Procion.sup.R -Red Agarose-purified IL 2 produced in the presence or absence of Daudi cells. The IL 2 preparations were treated with 2% sodium dodecyl sulfate and 5 mM 2-mercaptoethanol and applied to a 15% polyacrylamide **gel**. After electrophoresis, the **gel** was sliced into 1 mm sections and proteins eluted with 0.3 ml phosphate-buffered saline (pH 7.2). The eluted material was. . .

DRWD FIG. 8 relates to the **gel** filtration chromatography of Blue Agarose-purified IL 2 on high performance liquid chromatography in the presence and absence of sodium dodecyl. . . native or treated with 1%

sodium dodecyl sulfate and 10 mM dithiothreitol, was applied to a high performance liquid chromatography **gel** filtration column. The following protein standards were used: bovine serum albumin (MW 68,000), ovalbumin (MW 43,000), chymotrypsinogen (MW 25,000), and. . .

DETD . . . dialyzed ammonium sulfate precipitate. After 30 minutes the diethylaminoethyl cellulose was spun down and the supernatant saved (Supernatant 1). The **pellet** was resuspended in 300 ml of 0.05M Tris-HCl (pH 7.8) containing 0.1M NaCl. After 10 minutes the diethylaminoethyl cellulose was. . .

DETD **Gel** Filtration (Fraction IV)

DETD . . . . al. (1951) J. Biol. Chem. 193:265]. For protein concentrations lower than 5 micrograms/ml, samples were subjected to sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis; the protein bands were visualized by the silver staining technique [Merril, C. R., (1979) Supra]; and the protein concentration. . .

DETD Sodium Dodecyl Sulfate-Polyacrylamide **Gel** Electrophoresis

DETD The discontinuous Tris-glycine system of Laemmli [Laemmli, U.K., (1970) 227:680] was used for 1.5-mm thick slab **gels** using a 5-20% gradient or a 15% of acrylamide. The samples were analyzed under both reduced (2% sodium dodecyl sulfate, 5% mercaptoethanol) and non-reduced (2% sodium dodecyl sulfate) conditions. After electrophoresis, **gels** were stained with Coomassie Brilliant Blue or by a silver nitrate method [Merril, C.R., et al., (1979) Supra]. Apparent molecular.

. . 68,000), ovalbumin (MW 43,000), carbonic anhydrase (MW 30,000), soybean trypsin inhibitor (MW 20,000) and alpha-lactalbumin (MW 14,500).

After electrophoresis, the **gels** were sliced into 1-mm sections and proteins from each slice were eluted in 0.3 ml phosphate-buffered saline (pH 7.2). After. . .

DETD . . . to O'Farrell [(1975) J. Biol. Chem. 250:4007], with 3/10 Biolyte (Bio-Rad) in the first dimension and a 10% acrylamide uniform **gel** in the second dimension. Isoelectric focusing was at 500 V for 20 hr; slab **gels** were run at 20 mA/**gel**.

DETD Staining: To stain the **gels** with silver (2) they are fixed in 50% methanol/12% acetic acid for 30 min (**gels** can be stored overnight in this solution). The **gels** tend to shrink in the 50% methanol solution. To expand them prior to staining, they are placed

in 10% ethanol/5% acetic acid for 2 hr, followed by three washes with 10% ethanol (5 min each). The **gels** are then soaked in 4% (wt/vol) paraformaldehyde/1.43% (wt/vol) sodium cacodylate (adjusted to pH 7.3 with HCl) for 30 min., followed by three 5-min washes with 10% ethanol. The **gels** are then agitated gently for 30 min in a cupric nitrate/silver nitrate solution (made by dissolving 3.5 g of silver. . .

DETD Next the **gels** are placed in fresh diammine solution (made within 5 min of use) prepared by mixing together 30 ml of a. . . remaining after the procedure must be discarded because an explosive complex may form upon storage! After the diammine rinse, the **gels** were washed for 1 min in a reducing solution containing 2.5 ml of 10% formaldehyde (10 ml of commercial formaldehyde. . . appear as brown or black spots at any time in the reducing solutions. Staining can be stopped by washing the **gel** in successive changes of deionized water. Image formation in the diammine step may occur if reagent-grade absolute ethanol and fresh. . . washing the glass slab plates thoroughly, immediately after each electrophoresis run, and using well washed surgical gloves when handling the **gels**. The **gels** are fragile after staining and should be photographed for a permanent record.

DETD **Gels** that are overdeveloped may be lightened with a photographic reducer such as the copper reducer of Smith [Walls, E. J.,. . . . sodium thiosulfate in 1 liter of water. Usually a 3:1 dilution of water to fresh reducer is used to lighten **gels**. The reduction is stopped by washing the **gel** in water.

DETD Stained **gels** may be kept in water. **Gels** that are to be dried for storage or autoradiography should be first soaked in 30% (wt/vol) sodium thiosulfate for 15 min followed by four 15-min water rinses. The **gels** should then be soaked for 5 min in a preserving solution [methanol/H.sub.2 O/glycerol, 70:27:3 (vol/vol) [Mayer J. W., (1976) Anal.. . .

DETD Autoradiography: **Gels** that were to be autoradiographed were dried as described above and then placed in x-ray film cassette holders (Kodak X-omatic,. . .

DETD . . . requires centrifugation to remove the Enzymobead Reagent from the reaction mixture, followed by immediate removal of the supernatant for subsequent **gel** filtration. The second method used for IL 2 utilizes direct application of the test tube mixture to a **gel** filtration column.

DETD In both cases, a Bio-**Gel** P-6 DG column is recommended for the separation of the unbound iodide from the labeled protein.

DETD . . . concentrations, as shown in FIG. 4. The 0.7M-0.8M NaCl eluate pool was found, through silver staining of a 5-20% gradient **gel** (sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis), to contain three molecular components with molecular weights of 14,500.+-.2000, 16,000.+-.1000 and 17,000.+-.1000 daltons depending on the experimental condition. . .

DETD The IL 2 preparation from various steps of purification were subjected to sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis analysis. Preparations obtained prior to the Blue Agarose chromatography (Fractions I-IV) were analyzed on a 5-20% gradient **gel** followed by Coomassie brilliant blue staining as shown in FIG. 5. Preparations obtained after Blue Agarose chromatography and Procion.sup.R -Red Agarose chromatography were also analyzed on a 5-20% gradient **gel** followed by the highly sensitive silver staining method as shown in FIG. 6.

DETD To obtain a better resolution, the purified IL 2 was also analyzed on a 15% acrylamide **gel**. After staining, a molecular weight pattern similar to that obtained in the gradient **gel** was found. A parallel **gel** was sliced into 1-mm sections and proteins from each slice were eluted in phosphate-buffered saline (pH 7.2). IL 2 activity. . .

DETD . . . dodecyl sulfate and 20 mM dithiothreitol at 37.degree. C. for 1

hour and applied to an high performance liquid chromatography **gel** filtration column. The column was eluted with buffer containing 0.1% sodium dodecyl sulfate and 1mM dithiothreitol. As shown in FIG.. . .

DETD . . . which contaminate most partially purified IL 2 preparations. For example, alpha-Interferon co-purified with IL 2 during ion exchange chromatography and **gel** filtration steps, but was clearly separated from IL 2 by Blue Agarose chromatography. See FIG. 3. After chromatography on Procion.sup.R. . .

DETD . . . sulfate, the 26,000-dalton IL 2 of the invention exhibited a molecular weight of 16,000-18,000 daltons by high performance liquid chromatography **gel** filtration. Sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of this denatured form demonstrated the presence of two biologically active bands with molecular weights of about 16,000 and. . .

DETD . . . a specific activity of 10.sup.6 U/mg of protein, and consists of two active bands on a silver-stained sodium dodecyl sulfate-polyacrylamide **gel** [Welte, K, et al. (1982) Supra].

DETD . . . patients received only preparative chemotherapy with cyclophosphamide (50 mg/kg for 4 days), cytosine arabinoside (200 mg/kg/day for 5 days) and 6-**thioguanine** (200 mg/kg/day for 5 days), whereas 2 other patients were conditioned similar to patients with leukemias but with less TBI. . . and weekly thereafter to day 100. All immune suppressive drugs were stopped at that time. Patients with graft- versus-host disease (**GvHD**) of at least grade 2 were treated with high dose prednisone (2 mg/kg/d). One AML patient received prednisone plus cyclosporine A (10 mg/kg/d) while another ALL patient was maintained only on **azathioprine** (50 mg/d). At the time of the IL2 analysis, 13 patients had **GvHD** (4 patients grade 1;3 patients grade 2; and 6 patients grade 3). Four of the 13 patients had acute **GvHD**.

DETD The Effect of **GvHD** and Immunosuppressive Drugs on Mitogen Responses to OKT3 Antibody: The study population consisted of three groups with respect to immunosuppressive. . . given no further immunosuppressive therapy after BMT. All patients receiving allogeneic BMT, were treated with prophylactic methotrexate, while those with **GvHD** received, in addition, high dose prednisone (see above). Only one patient received prednisone plus cyclosporine A for **GvHD**. No differences between groups were seen with respect to endogenous IL2 production (Table XIV) and proliferative responses to OKT3 antibody. . .

DETD The study group included 13 patients who developed acute or chronic **GvHD** (grade 1-3) (shown with asterisks beside the symbols in FIG. 1). There were no statistically significant differences in the mitogen responses nor in the restoration of proliferation of PBMC by hpIL2 between patients with or without **GvHD**.

DETD . . . to PHA. IL2 has previously been shown to be able to restore (a) impaired cell-mediated lympholysis in patients with acute **GvHD** but not chronic **GvHD** [Mori, T, et al. (1983) J. Immunol. 130:712] and (b) PHA stimulated T cell colony-formation of lymphocytes from patients early. . .

DETD . . . Molecular and Cellular Biology, Steamboat Springs 1983 (in press)]. However, in patients after BMT IL2 might enhance or cause acute **GvHD**. Animal studies have been initiated to address this problem.

CLM What is claimed is:

. . . the group consisting of interleukin-2s characterized by molecular weights of about 14,500.+-.2,000, 16,000.+-.1,000, 17,000.+-.1,000, and

26,000.+-.4,000 daltons as measured by **gel** filtration and sodium dodecyl sulfatepolyacrylamide **gel** electrophoresis.

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. . . the group consisting of interleukin-2s characterized by molecular weights of about 14,500.+-.2,000, 16,000.+-.1,000, 17,000.+-.1,000, and 26,000.+-.4,000 daltons as measured by **gel** filtration and sodium dodecyl sulfatepolyacrylamide **gel** electrophoresis.

. . . the group consisting of interleukin-2s characterized by molecular weights of about 14,500.+-.2,000, 16,000.+-.1,000, 17,000.+-.1,000, and 26,000.+-.4,000 daltons as measured by **gel** filtration and sodium dodecyl sulfatepolyacrylamide **gel** electrophoresis.

L9 ANSWER 67 OF 68 USPATFULL

AB Interleukin 2 (IL 2; T-cell growth factor), produced with and without costimulation by Burkitt's lymphoma line Daudi, is highly purified approximately over 37,000-fold to apparent homogeneity from lymphocyte-conditioned medium derived from normal human blood cells by (NH.sub.4).sub.2 SO.sub.4 -precipitation, ion-exchange chromatography, **gel** filtration and hydrophobic chromatography. hp IL-2 is free of pyrogens, B cell inducing factor, B cell growth factor, interferon, CSF, and thymocyte differentiating factor. Nature IL 2 produced in the absence of Daudi cells has a molecular weight of about 26,000 daltons as measured by **gel** filtration and yields IL 2 having two molecular weights of about 16,000 and 17,000 daltons after denaturation as measured by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. IL 2 produced in the presence of Daudi cells shows a molecular weight of approximately 14,500 daltons as measured by both **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis.

This highly purified IL 2 is shown to correct immunodeficiency states in vitro and in vivo, especially in human patients. It shows value in the treatment of AIDS and immunodeficiency resulting from chemotherpy of cancer, as well as transplantation disorders such as graft versus host disease.

AN 88:67475 USPATFULL
TI Highly purified human interleukin 2 and method
IN Mertelsmann, Roland, Chappaqua, NY, United States
Welte, Karl, New York, NY, United States
Venuta, Salvatore, Napoli, Italy
PA Sloan-Kettering Institute for Cancer Research, New York, NY, United States (U.S. corporation)
PI US 4778879 19881018 <--
AI US 1984-603580 19840425 (6)
RLI Continuation-in-part of Ser. No. US 1982-370223, filed on 20 Apr 1982, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Foelak, Morton; Assistant Examiner: Nutter, Nathan M.
LREP White, John P.
CLMN Number of Claims: 1

ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 3136
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 4778879 19881018 <--
AB . . . over 37,000-fold to apparent homogeneity from lymphocyte-conditioned medium derived from normal human blood cells by (NH.sub.4).sub.2 SO.sub.4 -precipitation, ion-exchange chromatography, **gel** filtration and hydrophobic chromatography. hp IL-2 is free of pyrogens, B cell inducing factor, B cell growth factor, interferon, CSF,. . . IL 2 produced in the absence of Daudi cells has a molecular weight of about 26,000 daltons as measured by **gel** filtration and yields IL 2 having two molecular weights of about 16,000 and 17,000 daltons after denaturation as measured by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. IL 2 produced in the presence of Daudi cells shows a molecular weight of approximately 14,500 daltons as measured by both **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis.
SUMM . . . line Daudi, is purified approximately 37,000-fold to apparent homogeneity from lymphocyte-conditioned medium by (NH.sub.4).sub.2 SO.sub.4 -precipitation, ion-exchange chromatography (diethylaminoethyl cellulose), **gel** filtration (AcA 44 Ultrogel), and hydrophobic chromatography, preferably on Blue Agarose and on Procion.RTM.-Red Agarose. IL2 can also be separated. . .
SUMM . . . 27 84
precipitate
III
DEAE cellu-
135 183,000
1,356 50 62
lose(DE 52)
IV
AcA 44 Ultro-
40 145,000
3,625 135 49
gel
V Blue Agarose
0.96 87,680
91,333 3,382 30
VI
Procion.sup.R -Red
0.055.sup.++
55,229
1,004,164
37,191
19
Agarose*

.sup.+ The IL 2 activity. . .
SUMM . . . IL 2 produced in the absence of Daudi cells has a molecular weight of about 26,000 daltons as measured by **gel** filtration and yields IL 2 having two molecular weights of about 16,000 and 17,000 daltons after denaturation as measured by sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. IL 2 produced in the presence of Daudi cells (10.sup.6 /ml) shows a molecular weight of approximately 14,500 daltons as measured by both **gel** filtration and sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis.
SUMM . . . and was free of any contaminating proteins as judged by silver

staining and by I.sup.125 exolabelling in sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis. It is also pyrogen-free as tested in rabbits. In this test doses of purified IL 2 were used comparable. . .

SUMM The work of Mier et al. [J. Immunology (1982) 128:1122] uses preparative **gel** electrophoresis so differs from the invention detailed herein. No use of Sendai virus or Daudi cells is found in Mier. . .

SUMM . . . over an anion-exchange chromatographic column (diethylaminoethyl-Sepharose). IL 2 activity eluted as a broad peak centered at approximately 0.07M NaCl. Subsequent **gel** filtration with an Ultrogel AcA 54 column separated the IL 2 from most of the detectable proteins, and this sequence. . . specific activity over the IL 2 in the serum-free lymphocyte-conditioned medium. The IL 2-containing material was further purified using polyacrylamide **gel** electrophoresis containing sodium dodecyl sulfate. The IL 2 activity corresponded to a pair of protein bands present in the 13,000 molecular weight region in the sodium dodecyl sulfate **gel**. This procedure has been reported by Mier et al. (1982) Supra and Frank et al., (1981) J. Immunol. 127:2361, for. . .

SUMM . . . The highly purified IL 2 obtained by us appears to be free of any contaminating proteins in sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis after staining with a silver nitrate method [Merril, C. R., et al. (1979) Proc. Nat'l. Acad. Sci. U.S.A. 76:4335),. . .

SUMM Up to three functionally active bands were detected in this preparation. Elution of the materials from the sliced **gels** possessed high specific activity. We found that the molecular species of IL 2 are dependent on the experimental conditions used. . .

SUMM . . . proteins from desired proteinaceous material by anion exchange; effecting separation by molecular weight of the IL 2-containing proteinaceous material by **gel** filtration; and separating IL 2, which is highly hydrophobic, from other lymphokines of about the same molecular weight via hydrophobic. . .

DRWD FIG. 2 concerns **gel** filtration of IL 2 on AcA 44 Ultrogel. DE 52-purified IL 2 was loaded on an AcA 44 Ultrogel column. . .

DRWD FIG. 5 illustrates a sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis profile of various steps of IL 2 purifications ((a) molecular weight standards: phosphorylase b (MW 94,000), bovine serum albumin. . . ammonium sulfate precipitate; (d) pool of IL 2-containing diethylaminoethyl cellulose eluate; (e) IL 2-containing fractions pooled from AcA 44 Ultrogel **gel** filtration).

DRWD FIG. 6 shows the sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of Blue Agarose- and Procion.RTM.-Red Agarose-purified IL 2. IL 2 was treated with 2% sodium dodecyl sulfate and 5 mM 2-mercaptoethanol and applied to a 5-20% gradient **gel**. The protein bands were visualized by a silver nitrate method. The following marker proteins (200 ng each) were used: ovalbumin. . .

DRWD FIG. 7 shows the IL2 activity of 1 mm **gel** slices after sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of Procion.RTM.-Red Agarose-purified IL 2 produced in the presence or absence of Daudi cells. The IL 2 preparations were treated with 2% sodium dodecyl sulfate and 5 mM 2-mercaptoethanol and applied to a 15% polyacrylamide **gel**. After electrophoresis, the **gel** was sliced into 1 mm sections and proteins eluted with 0.3 ml phosphate-buffered saline (pH 7.2). The eluted material was. . .

DRWD FIG. 8 relates to the **gel** filtration chromatography of Blue Agarose-purified IL 2 on high performance liquid chromatography in the

presence and absence of sodium dodecyl. . . native or treated with 1%
sodium dodecyl sulfate and 10 mM dithiothreitol, was applied to a high performance liquid chromatography **gel** filtration column. The following protein standards were used: bovine serum albumin (MW 68,000),
ovalbumin (MW 43,000), chymotrypsinogen (MW 25,000), and. . .
DETD . . . dialyzed ammonium sulfate precipitate. After 30 minutes the diethylaminoethyl cellulose was spun down and the supernatant saved (Supernatant 1). The **pellet** was resuspended in 300 ml of 0.05M Tris-HCl (pH 7.8) containing 0.01M NaCl. After 10 minutes the diethylaminoethyl cellulose was. . .
DETD **Gel** Filtration (Fraction IV)
DETD . . . (1951) J. Biol. Chem. 193: 265]. For protein concentrations lower than 5 micrograms/ml, samples were subjected to sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis; the protein bands were visualized by the silver staining technique [Merril, C. R., (1979) Supra]; and the protein concentration. . .
DETD Sodium Dodecyl Sulfate-Polyacrylamide **Gel** Electrophoresis
DETD The discontinuous Tris-glycine system of Laemmli [Laemmli, U.K., (1970) 227: 680] was used for 1.5-mm thick slab **gels** using a 5-20% gradient or a 15% of acrylamide. The samples were analyzed under both reduced (2% sodium dodecyl sulfate, 5% mercaptoethanol) and non-reduced (2% sodium dodecy sulfate) conditions. After electrophoresis, **gels** were stained with Coomassie Brilliant Blue or by a silver nitrate method [Merril, C. R., et al., (1979) Supra]. Apparent. . . 68,000), ovalbumin (MW 43,000), carbonic anhydrase (MW 30,000), soybean trypsin inhibitor (MW 20,000) and alpha-lactalbumin (MW 14,500). After electrophoresis, the **gels** were sliced into 1-mm sections and proteins from each slice were eluted in 0.3 ml phosphate-buffered saline
(pH 7.2). After. . .
DETD . . . O'Farrell [(1975) J. Biol. Chem. 250: 4007], with 3/10 Biolyte (Bio-Rad) in the first dimension and a 10% acrylamide uniform **gel** in the second dimension. Isoelectric focusing was at 500 V for 20 hr; slab **gels** were run at 20 mA/**gel**.
DETD Staining: to stain the **gels** with silver (2) they are fixed in 50% methanol/12% acetic acid for 30 min (**gels** can be stored overnight in this solution). The **gels** tend to shrink in the 50% methanol solution. To expand them prior to staining, they are placed
in 10% ethanol/5% acetic acid for 2 hr, followed by three washes with 10% ethanol (5 min each). The **gels** are then soaked in 4% (wt/vol) paraformaldehyde/1.43% (wt/vol) sodium cacodylate (adjusted to pH 7.3 with HCl) for 30 min., followed by three 5-min washes with 10% ethanol. The **gels** are then agitated gently for 30 min in a cupric nitrate/silver nitrate solution (made by dissolving 3.5 g of silver. . .
DETD Next the **gels** are placed in fresh diammine solution (made within 5 min of use) prepared by mixing together 30 ml of a. . . remaining after the procedure must be discarded because an explosive complex may form upon storage!. After the diammine rinse, the **gels** were washed for 1 min in a reducing solution containing 2.5 ml of 10% formaldehyde (10 ml of commercial formaldehyde. . . appear as brown or black spots at any time in the reducing solutions. Staining can be stopped by washing the **gel** in successive changes of deionized water. Image formation in the diammine step may occur if reaent-grade absolute ethanol and fresh. . . washing the glass slab plates thoroughly, immediately after eacch electrophoresis run, and using well washed surgical glovres when handling the **gels**. The

gels are fragile after staining and should be photographed for a permanent record.

DETD **Gels** that are overdeveloped may be lightened with a photographic reducer such as the copper reducer of Smith [Walls, E. J.,.

. . sodium thiosulfate in 1 liter of water. Usually a 3:1 dilution of water to fresh reducer is used to lighten **gels**. The reduction is stopped by washing the **gel** in water.

DETD Stained **gels** may be kept in water. **Gels** that are to be dried for storage or autoradiography should be first soaked in 30% (wt/vol) sodium thiosulfate for 15 min followed by four 15-min water rinses. The **gels** should then be soaked for 5 min in a preserving solution [methanol/H.sub.2 O/glycerol, 70:27:3 (vol/vol) [Mayer J. W., (1976) Anal.. . .

DETD Autoradiography: **Gels** that were to be autoradiographed were dried as described above and then placed in x-ray film cassette holders (Kodak X-omatic,. . .

DETD . . . requires centrifugation to remove the Enzymobead Reagent from the reaction mixture, followed by immediate removal of the supernatant for subsequent **gel** filtration. The second method used for IL2 utilizes direct application of the test tube mixture to a **gel** filtration column.

DETD In both cases, a Bio-**Gel** P-6 DG column is recommended for the separation of the unbound iodide from the labeled protein.

DETD . . . concentrations, as shown in FIG. 4. The 0.7M-0.8M NaCl eluate pool was found, through silver staining of a 5-20% gradient **gel** (sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis), to contain three molecular components with molecular weights of 14,500.+-.2000, 16,000+1000 and 17,000.+-.1000 daltons depending on the experimental condition. . .

DETD The IL 2 preparation from various steps of purification were subjected to sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis analysis. Preparations obtained prior to the Blue Agarose chromatography (Fractions I-IV) were analyzed on a 5-20% gradient **gel** followed by Coomassie brilliant blue staining as shown in FIG. 5. Preparations obtained after Blue Agarose chromatography and Procion.sup.R -Red Agarose chromatography were also analyzed on a 5-20% gradient **gel** followed by the highly sensitive silver staining method as shown in FIG. 6.

DETD To obtain a better resolution, the purified IL 2 was also analyzed on a 15% acrylamide **gel**. After staining, a molecular weight pattern similar to that obtained int he gradient **gel** was found. A parallel **gel** was sliced into 1-mm sections and proteins from each slice were eluted in phosphate-buffered saline (pH 7.2). Il 2 activity. . .

DETD . . . dodecyl sulfate and 20 mM dithiothreitol at 37.degree. C. for 1 hour and applied to an high performance liquid chromatography **gel** filtration column. The column was eluted with buffer containing 0.1% sodium dodecyl sulfate and 1 mM dithiothreitol. As shown in. . .

DETD . . . which contaminate most partially purified IL 2 preparations. For example, alpha-Interferon co-purified with IL 2 during ion exchange chromatography and **gel** filtration steps, but was clearly separated from IL 2 by Blue Agarose chromatography. See FIG. 3. After chromatography on Procion.RTM.-Red. . .

DETD . . . sulfate, the 26,000-dalton IL 2 of the invention exhibited a molecular weight of 16,000-18,000 daltons by high performance liquid

chromatography **gel** filtration. Sodium dodecyl sulfate-polyacrylamide **gel** electrophoresis of this denatured form demonstrated the presence of two biologically active bands with molecular weights of about 16,000 and. . .

DETD . . . . a specific activity of 10.sup.6 U/mg of protein, and consists of two active bands, on a silver-stained sodium dodecyl sulfate-polyacrylamide **gel** [Welte, K, et al. (1982) Supra].

DETD . . . patients received only preparative chemotherapy with cyclophosphamide (50 mg/kg for 4 days), cytosine arabinoside (200 mg/kg/day for 5 days) and 6-**thioguanine** (200 mg/kg/day for 5 days), whereas 2 other patients were conditioned similar to patients with leukemias but with less TBI. . . and weekly thereafter to day 100. All immune suppressive drugs were stopped at that time. Patients with graft- versus-host disease (**GvHD**) of at least grade 2 were treated with high dose prednisone (2 mg/kg/d). One AML patient received prednisone plus cyclosporine A (10 mg/kg/d) while another ALL patient was maintained only on **azathioprine** (50 mg/d). At the time of the IL2 analysis, 13 patients had **GvHD** (4 patients grade 1; 3 patients grade 2; and 6 patients grade 3). Four of the 13 patients had acute **GvHD**.

DETD The Effect of **GvHD** and Immunosuppressive Drugs on Mitogen Responses to OKT3 Antibody: The study population consisted of three groups with respect to immunosuppressive. . . given no further immunosuppressive therapy after BMT. All patients receiving allogeneic BMT, were treated with prophylactic methotrexate, while those with **GvHD** received, in addition, high dose prednisone (see above) Only one patient received prednisone plus cyclosporine A for **GvHD**. No differences between groups were seen with respect to endogenous IL2 production (Table XIV) and proliferative responses to OKT3 antibody. . .

DETD The study group included 13 patients who developed acute or chronic **GvHD** (grade 1-3) (shown with asterisks besides the symbols in FIG. 1). There was no statistically significant differences in the mitogen responses nor in the restoration of proliferation of PBMC by hpIL2 between patients with or without **GvHD**.

DETD (a) . . . to PHA. IL2 has previously been shown to be able to restore impaired cell-mediated lympholysis in patients with acute **GvHD** but not chronic **GvHD** [Mori, T., et al. (1983) J. Immunol. 130: 712] and (b) PHA stimulated T cell colony-formation of lymphocytes from patients. . .

DETD . . . Molecular and Cellular Biology, Steamboat Springs 1983 (in press)]. However, in patients after BMT IL2 might enhance or cause acute **GvHD**. Animal studies have been initiated to address this problem.

L9 ANSWER 68 OF 68 USPATFULL

AB Method of treating autoimmune diseases such as rheumatoid arthritis by administration of a suppressor factor obtained in the supernatant of a human cell line. A particular human cell line is CEM which has survived treatment with 6-**thioguanine**.

AN 87:77878 USPATFULL

TI Treatment of autoimmune diseases such as rheumatoid arthritis with suppressor factor

IN Lau, Catherine Y., Unionville, Canada

PA Ortho Pharmaceutical (Canada) Ltd., Canada (non-U.S. corporation)

PI US 4705687 19871110 <--

AI US 1985-745116 19850617 (6)

DT Utility

FS Granted
EXNAM Primary Examiner: Brown, Johnnie R.; Assistant Examiner: Rollins, Jr., John W.
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 617
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 4705687 19871110 <--
AB . . . in the supernatant of a human cell line. A particular human cell line is CEM which has survived treatment with 6-**thioguanine** . .
SUMM . . . including dermatitis and aplastic anemia. Dramatic improvements in rheumatoid arthritis have been seen with immunosuppressive agents such as chlorambucil, cyclophosphamide, **mercaptopurine** and **azathioprine**. However, such drugs are associated with serious toxicity, may be teratogenic and may cause increases in lymphoma and infection. For. . .
SUMM . . . asthma, functional autonomic abnormalities, juvenile insulin-dependent diabetes. Pernicious anemia, Addison's disease, idiopathic hypoparathyroidism, spontaneous infertility, premature ovarian failure, pemphigus, bullous **pemphigoid**, primary biliary cirrhosis, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura and idiopathic neutropenia.
SUMM (i) is secreted by a stable 6-**thioguanine**-resistant mutant of the lymphoblastoid cell line CEM.
SUMM . . . from the supernatant produced by those cells of the CEM lymphoblastic leukemic cell line which are resistant to destruction by 6-**thioguanine** as described in the following preparations A, B, C and D and in my co-pending U.S. patent application Ser. No.. . .
SUMM The 6-thioquanine (2-amino-6-**mercaptopurine**: 6T) was obtained from Sigma (Cat. No. A-4882). 100 mg of 6T was dissolved in 100 ml of distilled water.. . .
SUMM TABLE 1

6-**Thioguanine** (.mu.g/ml)	% Cell Viability.sup.1
0.5	51
5	43
10	32
20	5
30	4

.sup.1 CEM cells were exposed to various concentrations of 6T for 7. . .
SUMM . . . dose range of 2-10.times.10.sup.4 units or 1-20 mg per kg of body weight until remission. SAF may be purified by **gel** filtration and ionic exchange chromatography or by SDS polyacrylamide electrophoresis until a single band is obtained. The specific activity of. . .